INHERITANCE OF RESISTANCE TO *XANTHOMONAS CAMPESTRIS* PV. *PRUNI* IN STEMS AND LEAVES OF JAPANESE-TYPE PLUMS FROM FOUR BREEDING POPULATIONS

By

BRUCE LEONARD TOPP

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1992

This is dedicated to my wife Thirley who seldom makes life easy, but always makes it worthwhile, to my children Matthew, Keenan, Nyssa and Lachlan of whom I am always proud, and to my parents who taught me the most important things I know. Above all else, I thank God for his love.

ACKNOWLEDGMENTS

I express my sincere thanks to Dr. Wayne Sherman for his guidance, encouragement and friendship, and for sharing with me his enthusiasm and skills in fruit breeding. He is a true gentleman. I thank Dr. Paul Lyrene for many hours of cosmopolitan discussion about genetics, evolution, and everything else. I also thank Dr. Robert Stall, Dr. Gloria Moore, Dr. Ken Quesenberry, Dr. Charles Wilcox, Dr. Tim White, Dr. Gary Hodge, Mr. Dudley Huber, Mr. Steve Linda and Mr. Jerry Minsavage for their advice and suggestions concerning my research, and for the generosity with which they have given their time.

I thank Dr. Dick Okie and Dr. David Ramming who generously provided germplasm from their breeding programs, and Mr. Dougal Russell who sent many parcels from Australia. Financial support from the Queensland Department of Primary Industries and moral support of Mr. Robin Barke are appreciated.

My family and I left Australia with some trepidation but have been overwhelmed with the generosity and helpfulness of the many new friends we have made in the United States. We will miss 'you all' and hope you can visit us sometime soon.

TABLE OF CONTENTS

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

INHERITANCE OF RESISTANCE TO *XANTHOMONAS CAMPESTRIS* PV. *PRUNI* IN STEMS AND LEAVES OF JAPANESE-TYPE PLUMS FROM FOUR BREEDING POPULATIONS

By

Bruce Leonard Topp

May, 1992

Chairman: Wayne B. Sherman
Major Department: Horticultural Science (Fruit Crops)

The inheritance of resistance to *Xanthomonas campestris* pv. *pruni* in leaves and stems of Japanese plum (*Prunus salicina*) was studied using half-sib families and their clonal seed parents sampled from plum breeding programs at California, Georgia, Florida, and Queensland. Parent trees and half-sib seedlings were evaluated for two years in a disease nursery after artificial inoculation and natural spread of disease. Populations from the four breeding programs differed in level of disease resistance. Georgia germplasm was most resistant averaging 24.2 spots per leaf and 13.3 mm cankers, and California germplasm was most susceptible

averaging 42.5 spots per leaf and 21.1 mm cankers. Broad sense heritabilities (H_B) were estimated from resistance measurements of grafted ramets of parent clones. For individual years, the H_B were between 0.3 and 0.6 for general leaf and stem resistance, but from a combined-years analysis the H_B were 0.331 ± 0.053 for number of spots per leaf, 0.206 ± 0.047 for leaf spot severity rating, 0.350 ± 0.054 for canker length, and 0.371 ± 0.054 for canker appearance rating. The lower H_B for combined years was due to genotype x year interaction which was higher for leaf resistance traits than for stem resistance traits.

Narrow sense heritabilities (h^2) were estimated by half-sib family variance component analysis for combined years as 0.128 ± 0.030 for number of spots per leaf, 0.248 ± 0.051 for leaf spot severity rating, 0.438 ± 0.068 for canker length, and 0.359 ± 0.062 for canker appearance rating. The moderate h^2 for stem canker traits indicates there will be adequate gain using recurrent mass selection. Genetic correlations were high (0.6 to 0.8) between leaf spot and stem canker resistance traits indicating there are common genes controlling these traits. Indirect selection for leaf spot resistance via selection for canker resistance would be an economical method of improving both traits.

Bacterial populations increased to high levels in leaves (10^8 cfu/cm^2 of leaf) and stems (10^7 cfu/10 mm stem piece) of susceptible clones. Resistance to stem cankers can be efficiently screened by inoculation of stems with 10^8 cfu/ml

inoculum and measurement of canker length after 6 weeks. Resistance to leaf spot that is associated with reduced bacterial levels can be effectively screened by leaf infiltration with 10^6 cfu/ml inoculum and bacterial population counts after 20 days.

CHAPTER 1
INTRODUCTION

Bacterial spot incited by *Xanthomonas campestris* pv. *pruni* (Smith) Dye is a major disease of Japanese plum (*Prunus salicina* L.) in many world locations (Anderson, 1956). Symptoms of the disease occur as angular spots on leaves, as greasy lesions which crack and develop into cankers on stems, and as star cracks on fruit (Heaton, 1983). Control of the disease on leaves, stems, and fruits is necessary for economical production of plums in locations of high disease incidence. Chemical control of bacterial spot is ineffective, and restrictions on chemical application to fruit trees are increasing so that breeding resistant plum genotypes is considered the best method of disease control (Heaton, 1983; Matthee, 1968). Fruit breeders require information on screening methods, inheritance of resistance traits, and a population with adequate genetic variation to efficiently select for disease resistance. Field evaluation of tree breeding germplasm is expensive due to large plant size (Hansche, 1983). Field screening for disease resistance using natural infection can be unreliable due to variation between years and locations. It is important to have reliable artificial inoculation and rating procedures for use in greenhouse and field. Studies of bacterial

populations in resistant and susceptible germplasm allow quantification of the host pathogen interaction and can aid in identifying resistant combinations (Graham et al., 1990, Hammerschlag, 1988a).

There have been no studies of quantitative genetics of *X. campestris* pv. *pruni* resistance in Japanese plum. Estimates of narrow sense heritabilities and the genetic correlations between leaf and stem resistance traits are needed to predict response to selection and to determine appropriate selection methods. Previous studies of bacterial spot resistance in plums have concentrated on rating commercially used clones (Du Plessis, 1988b; Keil and Fogle, 1974; Simeone, 1982, 1990). It would be of interest to examine a more diverse population of plums that are available in current breeding programs to quantify the variation and identify outstanding individuals (on phenotype) and parents (on combining ability).

The purpose of this program was to study the inheritance of resistance to *X. campestris* pv. *pruni* in a diverse collection of plum germplasm from four breeding programs. Specifically the objectives were to estimate genetic parameters that would allow quantification of response to selection, to determine efficient methods of artificially inoculating and rating leaf and stem resistance, and to examine the diversity of resistance available from the four populations.

CHAPTER 2

LITERATURE REVIEW

Symptoms and Etiology of Bacterial Spot on Plum

Bacterial spot of stone fruit caused by *Xanthomonas campestris* pv. *pruni* (Smith) Dye occurs frequently in summer rainfall regions (Anderson, 1956; Dunegan, 1932). The disease is called bacterial spot in reference to the lesions that occur on leaves and fruit of peach and nectarine. However, the name 'bacterial spot' is misleading when applied to plums because the cankers that develop on stems are a major part of the disease. Two types of stem cankers are differentiated depending on when they appear. Summer cankers develop on current season's growth from early to mid summer, whereas spring cankers appear in early spring on last season's wood that was infected the previous autumn (Thornberry and Anderson, 1933). Summer cankers begin as greasy lesions which elongate and become brown and sunken. Cracks form in the stem lesions and then develop into open cankers exuding gum and bacteria (Heaton, 1983). Leaf lesions appear as angular, watersoaked spots which dry to a light tan. Leaf spots commonly occur near the mid-vein and may coalesce and result in premature leaf

fall in severe cases (Moffett, 1973). Fruit lesions start as dark, circular, watersoaked spots which become sunken and may crack and ooze gum (Heaton, 1983).

Leaf infections occur in spring and continue throughout the growing season following periods of moist weather (Moffett, 1973). Inoculum each spring comes from bacteria surviving the winter months in stems (Dunegan, 1932; Moffett, 1973). During wet periods or heavy dews, bacteria are exuded mainly from newly formed spring cankers and dispersed to leaves and fruit by splashing water. Stems are invaded in autumn through fresh leaf scars and the bacteria survive winter in intercellular spaces of the cortex, phloem and xylem parenchyma (Feliciano and Daines, 1970). Summer stem cankers may also develop by systemic movement of the bacteria from infected leaves through petioles (Du Plessis, 1984, 1986, 1987a, 1988a). Summer cankers were not observed to form after January (late summer) in Queensland and new infections of fruit ceased 4 to 6 weeks before harvest (Moffett, 1973). Resistance of mature tissue to bacterial (*Xanthomonas campestris*) infection has also been noted in plum by Du Plessis (1987a) and in citrus (Stall et al., 1982).

Bacteria enter plants through stomata, lenticels, hydrathodes, or mechanically caused wounds (Huang, 1986). A continuous water column from the stomatal cavity to the leaf surface is necessary for capillary movement of epiphytic

bacteria into the leaf (Matthee, 1968). Bacterial spot has been noted more commonly on the windward side of trees (Matthee, 1968). Wind driven rains favor disease spread because of increased bacterial dissemination, watersoaking of leaves, and forcible removal of leaves allowing bacterial entry via fresh leaf scars (Dunegan, 1932; Feliciano and Daines, 1970).

Bacterial spot is worse on sandy soils than on heavy soils. Matthee and Daines (1968) suggested this was due to increased root aeration in sandy soils, which results in increased water congestion of leaves. Bacterial spot is also spread through vegetative propagation (Goodman and Hattingh, 1986) and by pruning (Goodman and Hattingh, 1988).

Resistance to *X. campestris* pv. *pruni* in *Prunus*

Resistance to *X. campestris* pv. *pruni* is a selection criterion in cultivar adaptation tests for peach and nectarine (Clayton, 1976; Sherman and Lyrene, 1981; Werner et al., 1986), apricot (Millikan and Hibbard, 1964), and Japanese plum (Russell et al., 1991; Simeone, 1990; Topp et al., 1989), as well as in Japanese plum breeding programs in Alabama (Norton, 1976), Florida (Sherman and Sharpe, 1970), Georgia (Thompson, 1981), Australia (Topp and Russell, 1988), and South Africa (Du Plessis, 1987b; Hurter and van Tonder, 1975). Cultivars and seedlings are generally screened after natural infection using leaf

spot severity rating scales, number of leaf spots per leaf or unit area, fruit spot severity rating scales, or percentage defoliation. Greenhouse inoculation systems have included immersion of leaves under vacuum (Daines and Hough, 1951), infiltration by high pressure sprays (Civerolo and Keil, 1976; Du Plessis, 1986), and infiltration by needleless syringe (Du Plessis, 1988b; Hammerschlag, 1988a; Randhawa and Civerolo, 1985). In vitro methods of resistance breeding in peach involve somaclonal variation, bacterial toxin selected cell cultures, and detached leaf and stem bioassays (Hammerschlag, 1988a, 1988b, 1990).

There have been no studies on inheritance of resistance to *X. campestris* pv. *pruni* in Japanese plum. Sherman and Lyrene (1981) concluded that leaf spot resistance in peach was controlled by a few major dominant genes due to the high level of resistance in populations that had not been selected for resistance. Layne (1966) reported that resistance to leaf spot and resistance to fruit spot in apricot may be inherited separately. Popenoe (1959) reported no good relation between susceptibility to stem cankers and susceptibility to fruit and leaf spots in Japanese plum. He also considered that stem canker resistance was associated with a spreading growth habit and hypothesized that erect growth and susceptibility were derived from *P. simonii*. *P. angustifolia* is a native plum of the southeastern United States where bacterial spot is endemic, and has been used as a source of resistance (Flory, 1941; Topp and Sherman, 1990).

Estimation of Quantitative Genetic Parameters

Quantitative characteristics that do not segregate in simple Mendelian ratios are commonly the traits that plant breeders are seeking to improve. Statistical methods have been developed which allow partitioning of the observed phenotypic variation into components due to additive effects of genes, non-additive effects of genes, and the environment (Crow, 1986; Hartl and Clark, 1989; Kempthorne, 1957). Further partitioning of environmental variation allows modification of experimental technique which may increase precision of measurement and reduce 'background noise' in the system (Gomez and Gomez, 1984). Broad sense heritability (H_B) is the ratio of genotypic to phenotypic variance. The H_B is deterministic in that it expresses the relative importance of genetic versus environmental variance and is estimated by repeated measurements on ramets of the one clone (Burton and DeVane, 1953) or by repeated measurements in time on individual ramets (Gordon, 1979). Crow (1986) suggests that H_B can also be considered as the regression of genotype on phenotype.

Narrow sense heritability (h^2) is defined as the ratio of additive genetic variance to phenotypic variance or equivalently as the regression of breeding value on phenotypic value (Falconer, 1989). Only the additive effects of genes (breeding values of the parent) are transferred from parents to offspring, not the dominance and epistatic effects which arise due to specific combinations of alleles

within and between loci. Estimates of h^2 are important in predicting response to selection because the choice of parents based on phenotype is only effective to the extent that the breeding value is measured by phenotypic value, i.e., response depends on the size of h^2 (Hansche, 1983).

Genetic parameters, including h^2, are estimated by:

1. Creating a set of relatives in a mating design,
2. Evaluating the relatives in an experimental design,
3. Estimating the statistical variance and covariance components described by Falconer (1989) as observational components of variance, and
4. Expressing the expectations of these variance components in terms of covariances among relatives and thus in terms of genetic variances.

These four steps apply equally to all methods of quantitative genetic parameter estimation and details of these methods are well documented by Becker (1985), Falconer (1989), and Hallauer and Miranda Fo (1981), or in more complex theoretical terms by Kempthorne (1957).

Two common methods of h^2 estimation that have particular relevance to Japanese plum are half-sib family partitioning of variance and parent offspring regression. Plums are generally self-infertile and cross-compatible and so half-sib

families are inexpensive to create using polycross, topcross or open pollination (Simmonds, 1979). Parent trees are routinely propagated by budding or grafting, and so can be reproduced for parent offspring experiments. Nguyen and Sleper (1983) provide an excellent review on the theory and application of h^2 estimation from half-sib families for forage grasses that is directly applicable to Japanese plums.

Inheritance studies of quantitative traits require large numbers of families to obtain precise estimates of heritability (Falconer, 1989; Klein et al., 1973). The resulting datasets are large and analyses are frequently complicated by non-orthogonality (Milliken and Johnson, 1984). The SAS (SAS, 1987) statistical software package allows analyses of complex random and mixed models via the GLM procedure but requires more memory than is available from current personal computers when there are more than 100 degrees of freedom in the model (programs are slow to run when degrees of freedom in the model exceed about 50). Large scale quantitative genetic experiments may therefore be difficult to analyze using SAS. Least Squares Maximum Liklihood Mixed and Weighted (LSMLMW) is a linear-models program written by Harvey (1990) with specific applications for quantitative geneticists in the animal sciences. LSMLMW employs an absorption procedure which allows solutions of extremely large models and will accommodate several hundred to a few thousand random effects

(Koonce, 1991). There are seven basic models but with multiple runs of the program other designs can be analyzed (Harvey, 1990). Koonce (1990) concluded that although LSMLMW is more difficult to use than SAS, it is useful for geneticists who routinely analyze unbalanced, messy datasets that conform to mixed models.

CHAPTER 3

COMPARISON OF GREENHOUSE METHODS FOR ASSESSING RESISTANCE TO BACTERIAL SPOT IN PLUM LEAF

Introduction

Field evaluation of fruit tree breeding germplasm is expensive due to large plant size and long juvenility period (Hansche, 1983). Selection methods which allow rouging of undesirable genotypes prior to field planting are therefore beneficial. Resistance to bacterial diseases can be assessed in this manner and a large number of methods have been used in various crops. Greenhouse systems for *Prunus* have included immersion of leaves under vacuum (Daines and Hough, 1951), infiltration by high pressure sprays (Civerolo and Keil, 1976; Du Plessis, 1986), and infiltration by needleless syringe (Randhawa and Civerolo, 1985; Du Plessis, 1988b; Hammerschlag, 1988b). The desirability of any one system of disease inoculation and assessment depends on its accuracy, precision, and correlation with field performance. Partitioning the total variance of random variables in experiments which compare plant genotypes allows determination of some of these factors (Campbell and Madden, 1990; Gomez and Gomez, 1984).

The purpose of this study was to compare four greenhouse leaf inoculation and assessment systems of *Xanthomonas campestris* pv. *pruni* (Smith) Dye on Japanese-type plums (*Prunus salicina* L.) for repeatability (precision), correlation with field ratings (accuracy), and ability to differentiate between genotypes.

Materials and Methods

Plant Material

Six plum genotypes were chosen at random from a large germplasm collection at Gainesville, Florida and propagated by stem cuttings to produce from four to seven ramets per genotype. Two ramets per genotype were grown in a greenhouse in 5 liter containers of a commercial potting mix (Terra-Lite Metro-mix 200, Cambridge, MA) for 12 months prior to inoculation. The remaining rooted cuttings were planted in the field in a disease nursery. An additional six genotypes were propagated in a similar manner for use in the correlation study but only one potted tree of each was assessed in the greenhouse.

Inoculum Preparation

A Florida isolate of *X. campestris* pv. *pruni* was grown overnight in Difco nutrient broth, pelleted by centrifugation, and resuspended in sterile, tap water to obtain 5 x 10^8 cfu/ml by photometrically standardizing to 0.3 A at 600-μm

wavelength. These suspensions were diluted serially with sterile tapwater to obtain the specified concentrations of inoculum.

Inoculation and Assessment Systems

All inoculations were performed in the greenhouse between 7:00 and 9:00 AM when the temperature was approximately 23°C. The temperatures during the fortnight of disease development ranged from 20°C to 35°C. Methods were as follows:

1. DIP - The apical 10 leaves of a branch were immersed in 2.5 x 10^8 cfu/ml inoculum and agitated for 5 seconds so that leaf surfaces were fully wetted. After 14 days the number of lesions per cm^2 leaf area was counted at four randomly selected sites on each of the three most severely affected leaves per branch.
2. CARB - The third, fourth, and fifth leaves from the growing tip were rubbed on the adaxial side with a slurry of carborundum powder and 2.5 x 10^8 cfu/ml inoculum. The number of lesions per cm^2 leaf were counted at four randomly selected sites on the three leaves 14 days after inoculation.
3. INF5 - The third, fourth, and fifth leaves from the growing tip were infiltrated with 5 x 10^5 cfu/ml inoculum with a needless syringe at

four sites per leaf to produce watersoaked circles of about 2 cm diameter. The watersoaking was not visible 1 hour after inoculation. The percentage of watersoaking and necrosis at each site was estimated 14 days after inoculation.

4. INF6 - Leaves were inoculated as described for INF5 but with 5 x 10^6 cfu/ml inoculum.

Experimental Design and Statistical Analysis

In the greenhouse, all four methods were applied to each tree with one branch randomly assigned for each method. The mathematical model was $Y_{ijkl}=\mu + G_i + P(G)_{ij} + L_k + LxG_{ik} + LxP(G)_{ijk} + E_{ijkl}$, where Y_{ijkl} is the disease assessment response of a single inoculation site on a leaf, μ is the overall mean, G_i is the effect of genotype, $P(G)_{ij}$ is the effect of plant nested within genotype, L_k is the fixed effect of leaf position, LxG_{ik} is the interaction of leaf position with genotype, $LxP(G)_{ijk}$ is the interaction of leaf position with plant nested within genotype, and E_{ijkl} is the residual error term. All effects are random except for leaf position which was considered fixed (Table 3.1). Analysis of variance and variance component estimates were obtained using the SAS GLM and VARCOMP procedures (SAS, 1987).

Repeatability was estimated using Fisher's intraclass correlation (Becker, 1985; Kempthorne, 1957) and standard errors of these variance ratios were derived as in Falconer (1989). Inspection of plots of residual versus predicted values indicated that transformations would aid in reducing dependence of the variance on the mean. Data for DIP, INF5 and INF6 were transformed by log (x+1) and for CARB by square root (x+1) prior to analysis of variance.

The relationship of greenhouse methods to field ratings was obtained by Pearson's interclass correlation via the SAS CORR procedure (SAS, 1987). Field ratings were taken in August 1991 in a disease nursery in which each tree was inoculated with *X. campestris* pv. *pruni* in June 1991 and disease spread was aided by the use of susceptible trees of 'Gulfruby' planted every 4 m between rows of test trees. The rating scale was a modification of the 0 to 5 scale used by Werner et al. (1986) where 0 = no symptoms, 1 = 0 to 1% of leaves with lesions, 2 = 1 to 5%, 3 = 5 to 15%, 4 = 15 to 40%, and 5 = over 40% of leaves with lesions. Field performance was based on the mean of 2 to 5 trees per genotype. The 0 to 5 ratings were converted to percentage values prior to calculating means based on the same reasoning that Elanco Tables are used to convert Horsfall-Barratt ratings (Campbell and Madden, 1990). Percentage values obtained in this manner were used for the Pearson and Spearman correlations.

Results and Discussion

Analysis of Greenhouse Methods

The proportion of variance attributable to differences between genotypes varied with disease assessment method (Table 3.2). CARB was highest, DIP and INF5 intermediate and INF6 was zero. INF6 did not detect significant differences between genotype resistances (Table 3.3). The 5 x 10^6 cfu/ml concentration of bacteria infiltrated into the leaf resulted in over 65% watersoaking in all genotypes (Table 3.4). Bacterial populations measured in 'Blackamber' and C333-1 (see Chapter 7) indicated there was no difference in pathogen development in these two genotypes once the bacteria were in the leaf mesophyll. It is possible that the inability of INF6 to distinguish differences among the genotypes is a true reflection of pathogen development and hence lack of variability in host resistance (Parlevliet, 1989). The plant within genotype component of variance was less than 15% of total variance for three of the methods and only for INF6 would it be advantageous to replicate by increasing the number of ramets per genotype. This finding is of particular relevance as these methods eventually will be used to screen seedling populations for bacterial leaf spot and replication of genotypes would add to the cost and time involved in testing.

Repeatability partitions the total variability in the experiment into a portion that arises due to differences between the genotype groups and a portion that

arises due to differences between members within each genotype group (i.e., into between group and within group components). It is appropriate in this study as it allows the comparison of the four different disease assessment methods even though they are measured in different units because, like the F test, it is a variance ratio and so is unitless. The methods DIP, measured in lesions per cm^2, and INF5, measured in percentage watersoaking, can thus be compared. Also, fruit breeders wish to use the inoculation and assessment system that differentiates between genotypes (increased σ^2_G) but minimizes the within genotype variance. This will be the method with highest repeatability.

The CARB method was most repeatable (0.63) followed by INF5 (0.39) and DIP (0.25) on the basis of one observation per leaf (Table 3.3). The INF6 method had a repeatability of zero because there was no variation between genotypes using this method, i.e., the σ^2_G was zero. However, the coefficient of variation for INF6 was 4.7 % indicating that this is a precise method of measurement.

Bacterial entry into the leaf by the DIP method depends on water congestion of intercellular spaces at the time of inoculation (Matthee and Daines, 1968) and on many environmental factors which influence bacterial longevity on the leaf surface (Hirano and Upper, 1983; Leben, 1974). In contrast, the infiltration methods place the bacteria into each leaf and so avoid these

environmental variations. These may be reasons for the lower repeatability of DIP compared to INF5. However, the infiltration methods also may avoid some plant epidermal resistance mechanisms and so less closely resemble natural field infection.

The CARB method appeared best for distinguishing among genotypes as measured by its F value of 20.3 (Table 3.3). The F value is misleading in this instance as it is a measure of the average difference between genotype means. The high F for CARB is due to a very large difference between one genotype and the other five genotypes. There was no significant difference among the remaining five genotypes (Table 3.4).

Correlations of Greenhouse Methods with Field Ratings

Pearson's correlation coefficients for DIP and CARB methods with field ratings were significant, but both infiltration method correlations were not significantly different from zero (Table 3.5, Figure 3.1). Spearman's correlation measures the correspondence of genotype rankings by the different methods and was not different from Pearson's correlation coefficient for DIP, INF5 and INF6 (Table 3.5). However the Spearman's correlation for CARB was only 0.289 compared with the Pearson's correlation of 0.644. This difference indicates that although the overall correspondence of CARB greenhouse ratings and field ratings

was relatively high, the ranking of clones in the greenhouse by CARB method did not correspond to the ranking of clones in the field (Figure 3.1B).

The DIP method had the highest correlations with field determined resistance, but a correlation of 0.691 accounts for only 47.8% of the variation between field and greenhouse resistance measurements. The DIP method may therefore be used as a preliminary screen when large populations are to be reduced in the greenhouse but field testing would be required as susceptible seedlings will be included in the selected group. For example if the threshold was set at four lesions per cm^2 in this experiment then two clones which average about 40% leaf infection in the field would be selected using the DIP method (Figure 3.1A). These would need to be culled during field screening.

The low correlations obtained in this study for the infiltration methods with field ratings are in contrast to reports by Randhawa and Civerolo (1985). They infiltrated detached peach leaves and reported a high degree of correspondence for 21 of the 22 peach genotypes tested, although no correlation was presented. Stall et al. (1982) also noted a good correspondence between leaf infiltration and field ratings for citrus canker but also that exceptions occurred. They considered that the outliers arose due to experimental technique which could be overcome with improved uniformity of leaf age and with use of several inoculum doses for each plant genotype.

The sample of genotypes involved in the study may partly explain the differences between studies. For example, when the outlier 'Bruce' in Figure 3.1C was excluded from the analysis, the correlation for INF5 with field rating rose to 0.88 ($P = 0.0004$). Greenhouse methods that correlate with field ratings to this extent would be extremely useful.

How could 'Bruce' be resistant in the field and yet susceptible when infiltrated with *X. campestris* pv. *pruni* in the greenhouse? Leaf infiltrations on 'Bruce' clones in the field were repeated (data not presented) to eliminate the possibility of error such as genotype misidentification. The resistant trees in the field gave similar watersoaking percentages to those obtained on clones in the greenhouse. Another possibility is that the field disease pressure was low enough to permit escapes. This may have been a possibility in a low bacterial spot year such as 1990, but rainfall was above average in 1991 and all four ramets of 'Bruce' rated resistant despite very high levels of bacterial spot in the disease nursery. Also 'Bruce' has been reported field resistant in Australia (Topp et al., 1989) and the United States (Keil and Ogle, 1974). Two other possible explanations which may be more likely as follows:

1. 'Bruce' has some level of epidermal resistance which results in resistant reactions in field ratings (and DIP method) but as susceptible reactions with infiltration methods.

2. 'Bruce' is exhibiting tolerance (Robinson, 1976) to the leafspot symptoms measured in the field, so that despite development of high bacterial concentrations in the leaf few leaf spots develop.

This could be tested by regressing bacterial population size against leaf spot development for a range of plum clones with deviants above the curve classed as tolerant. The high correlation (0.88) obtained for INF5 when 'Bruce' was excluded from the data indicates this may be a useful method of screening for resistance that is associated with low pathogen numbers rather than for resistance via tolerance or pathogen avoidance.

Summary and Conclusions

No greenhouse screening method was clearly superior in all categories of assessment. The CARB method, although the most repeatable (precise), is unsatisfactory because it did not rank genotypes as they performed in the field (Spearman's correlation of 0.289 ± 0.336). The INF6 method did not distinguish differences between the genotypes because all plants were susceptible in the mesophyll when infiltrated with 5×10^6 cfu/ml of inoculum. It is possible that the field rating may differentiate between levels of quantitative resistance that are not associated with large differences in bacterial populations in the leaf mesophyll, and hence, there were no differences in percentage watersoaking with INF6

despite the differences indicated by the field rating. DIP appears the most useful of the four methods tested, with a repeatability of 0.46 for four observations per leaf. The DIP method ranked the genotypes most closely with field performance (Spearman's correlation of 0.561 ± 0.248), and detected differences among the genotypes. Field screening under severe disease pressure provides the best measure of disease resistance, but ideal test environments are not available at all locations or in all years; they also suffer the problems of high cost due to land and time commitments in assessment. For these reasons a greenhouse method needs to be used and the best system in this study was the DIP greenhouse method. A greenhouse system that is more repeatable and correlates more highly with field results would be desirable.

Table 3.1. Sources of variation, degrees of freedom (df) and expected mean squares (EMS) for analysis of the greenhouse assessment methods DIP, INF5, INF6 and CARB used to determine bacterial leaf spot resistance.

Source	df	EMS
Genotype	5	$\sigma_e^2 + 12\,\sigma_{P(G)}^2 + 24\,\sigma_G^2$
Plant (G)	6	$\sigma_e^2 + 12\,\sigma_{P(G)}^2$
Leaf position	2	$\sigma_e^2 + 4\,\sigma_{P(G)xL}^2 + 8\,\sigma_{GxL}^2 + 48\,\Theta_L^2$
G x L	10	$\sigma_e^2 + 4\,\sigma_{P(G)xL}^2 + 8\,\sigma_{GxL}^2$
P(G) x L	12	$\sigma_e^2 + 4\,\sigma_{P(G)xL}^2$
Error	130	σ_e^2

Table 3.2. Variance component estimates (and percentage of total variance in parentheses) for the greenhouse assessment methods DIP, INF5, INF6 and CARB used to determine bacterial leaf spot resistance.

	Disease assessment method			
Source	DIP	INF5	INF6	CARB
Genotype	0.327 (25)	0.790 (39)	0 (0)	1.360 (63)
Plant (G)	0.133 (14)	0.144 (7)	0.020 (30)	0.018 (1)
G x L	0 (0)[z]	0.125 (6)	0.006 (9)	0 (0)
P(G) x L	0 (0)	0.316 (16)	0 (0)	0.215 (10)
Error	0.582 (61)	0.663 (32)	0.040 (61)	0.557 (26)

[z]Negative estimates of variance set to zero.

Table 3.3. Repeatability and genotype F test values for the greenhouse assessment methods DIP, INF5, INF6 and CARB used to determine bacterial leaf spot resistance.

Assessment method	Repeatability (± SE) 1 obs./leaf[z]	4 obs./leaf[y]	F test between genotypes[x]
DIP	0.25 ± 0.14	0.46 ± 0.16	3.78^{NS}
INF5	0.39 ± 0.17	0.51 ± 0.17	6.46*
INF6	0.00	0.00	0.47^{NS}
CARB	0.63 ± 0.14	0.79 ± 0.21	20.30**

^{NS},*,**Nonsignificant or significant at $P \leq 0.05$ and 0.01, respectively.

[z]Repeatability for 1 observation/leaf = $\dfrac{\sigma^2_G}{\sigma^2_G + \sigma^2_{P(G)} + \sigma^2_{LxG} + \sigma^2_{LxP(G)} + \sigma^2_e}$.

[y]Repeatability for 4 observations/leaf = $\dfrac{\sigma^2_G}{\sigma^2_G + \sigma^2_{P(G)} + \sigma^2_{LxG} + \sigma^2_{LxP(G)} + \sigma^2_e/4}$.

[x]F = Genotype Mean Square/Plant (G) Mean Square.

Table 3.4. Mean genotype disease response for the greenhouse assessment methods DIP, INF5, INF6 and CARB.

Genotype	Greenhouse screening method[z]			
	DIP (lesions per cm^2)	INF5 (% water-soaked)	INF6 (% water-soaked)	CARB (lesions per cm^2)
Gulfruby	6.375 a[y]	28.125 a	85.417 a	17.500 a
Blackamber	5.542 a	22.625 ab	83.750 a	4.042 b
C109-6	3.042 ab	12.750 bcd	65.625 a	2.250 b
C333-2	1.667 b	13.833 bc	85.294 a	1.208 b
C113-5	0.792 b	4.292 cd	75.000 a	1.083 b
C333-1	0.375 b	1.542 d	78.182 a	0.958 b

[z]Values are means of 24 observations per genotype (3 leaves x 4 sites/leaf x 2 plants) and are on untransformed data.

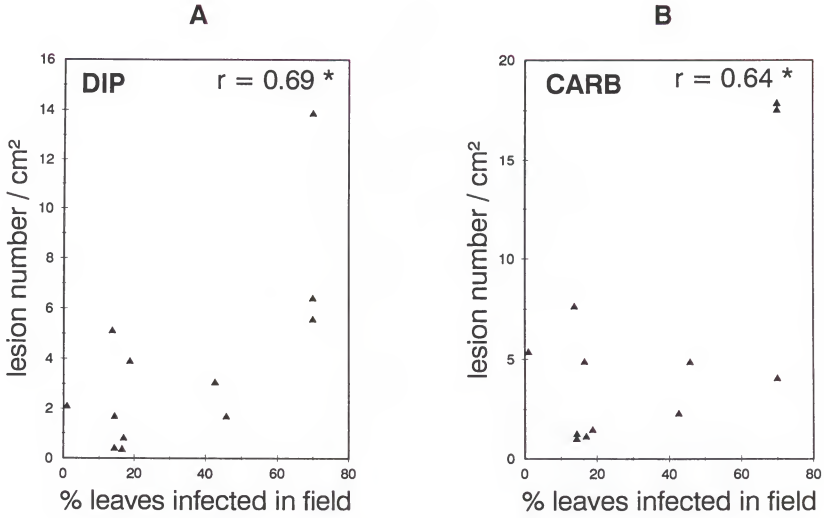
[y]Mean separation in columns by Duncan's multiple range test, $P \leq 0.05$.

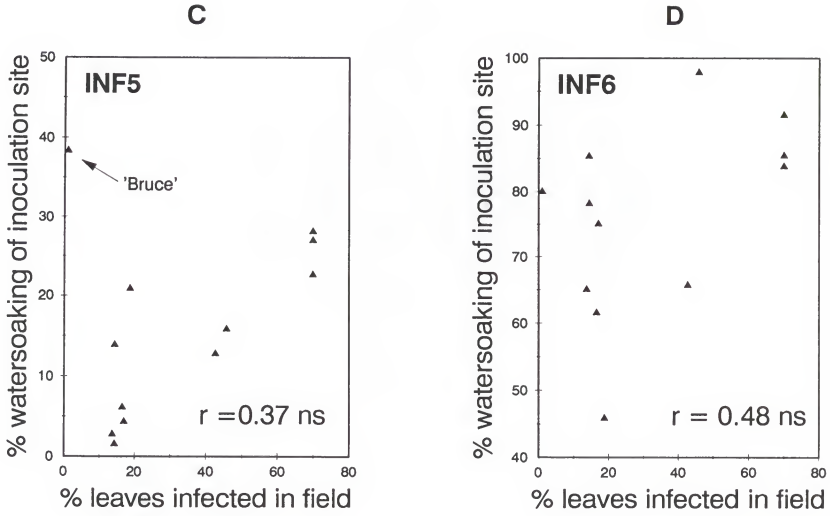
Table 3.5. Phenotypic correlations between genotype resistance ratings from DIP, INF5, INF6 and CARB greenhouse assessment methods and genotype resistance ratings from the field.

Greenhouse method	Pearson's correlation coefficient (± SE)	Spearman's correlation coefficient (± SE)
DIP	0.691 ± 0.096	0.561 ± 0.248
INF5	0.370 ± 0.338	0.444 ± 0.384
INF6	0.476 ± 0.133	0.465 ± 0.214
CARB	0.644 ± 0.175	0.289 ± 0.336

Figure 3.1. Scatterplots of 1991 field rating of percent of leaves with lesions versus greenhouse ratings from the four inoculation methods.

A) Field rating versus DIP.
B) Field rating versus CARB.
C) Field rating versus INF5.
D) Field rating versus INF6.


A
DIP
r = 0.69 *
lesion number / cm²
% leaves infected in field
B
CARB
r = 0.64 *
lesion number / cm²
% leaves infected in field


C
INF5
'Bruce'
r =0.37 ns
% watersoaking of inoculation site
% leaves infected in field
D
INF6
r = 0.48 ns
% watersoaking of inoculation site
% leaves infected in field

CHAPTER 4

ANALYSIS OF BACTERIAL SPOT RESISTANCE IN LEAVES AND STEMS OF PLUM CLONES FROM FOUR BREEDING PROGRAMS

Introduction

Bacterial spot incited by *Xanthomonas campestris* pv. *pruni* (Smith) Dye is a major disease of Japanese plum (*Prunus salicina* L.) in many world locations (Anderson, 1956). Selection for resistance to *X. campestris* pv. *pruni* is an objective of breeding programs in Australia, South Africa, Florida, Georgia, and Alabama (Hurter and van Tonder, 1975; Norton, 1976; Sherman and Lyrene, 1985; Thompson, 1981; Topp et al., 1989). Symptoms of the disease are expressed as angular spots on leaves, greasy lesions which crack and develop into cankers on stems, and as greasy and sunken spots which crack on the fruit (Heaton, 1983). Plum clones have been evaluated and ranked for resistance to bacterial spot (Du Plessis, 1988b; Keil and Fogle, 1974; Simeone, 1982, 1990). These studies have concentrated on commercially available cultivars which do not fully sample plum germplasm currently used in breeding. Also, the leaf component of the disease has been the main basis for ranking genotypes despite the importance of stem cankers in killing branches and providing a source of overwintering inoculum

(Moffett, 1973). The purpose of this study was to evaluate *X. campestris* pv. *pruni* resistance in both leaves and stems in a diverse collection of plum clones from breeding programs at California, Florida, Georgia, and Australia. Specifically, we compared leaf and stem resistance in material from the four breeding programs, compared the effect of year on expression of resistance, estimated broad sense heritabilities, and identified genotypes with potential as parents for future breeding.

Materials and Methods

Plant Material

Scionwood was collected of 66 plum genotypes (Table 4.1) used in breeding programs at the United States Department of Agriculture (USDA) Fresno, California; University of Florida, Gainesville, Florida; USDA Byron, Georgia; and Queensland Department of Primary Industries Applethorpe, Queensland, Australia, and budded onto peach seedling rootstock (cv. 'Nemaguard') in May 1989 to produce a total of 283 trees. The genotypes were random selections of the germplasm from the breeding programs and included genotypes bred at other centers but used as parents or being evaluated as potential parents in the programs.

An average of four ramets of each genotype was planted in a completely randomized design at Gainesville, Florida in January 1990, at spacings of 90 cm between rows and 60 cm between trees. Bacterial spot susceptible 'Gulfruby' trees were planted along the alleyways every 4 m to ensure even inoculum load. Trees were irrigated with overhead sprinklers and were not sprayed with bactericides. Trees were headed back to about 15 cm above ground level in winter to force vigorous new growth the following spring and to remove infected branches which may have provided uneven distribution of inoculum.

Inoculation and Rating Methods

Leaves were inoculated between 6:00 and 8:00 AM in June 1990 and May 1991 (early summer). Stems were inoculated on the same day as leaves but between 8:00 AM and 9:00 PM. One actively growing branch per tree was tagged and the top 10 leaves were immersed in 2.5×10^8 cfu per ml *X. campestris* pv. *pruni* inoculum and agitated for 5 seconds until leaf surfaces were fully wetted. The same branch was injected at three sites within the top 15 cm of stem using a 26 gauge needle and syringe and 2.5×10^8 cfu per ml inoculum. The number of lesions on the two leaves with highest incidence of disease were counted 14 days after inoculation. The average number of spots on these two leaves is the variable LS12.

Length of the three stem cankers was measured 6 weeks after inoculation. The average length (mm) of these three cankers is the variable CANKA. Cankers were rated at this time on a 1 to 5 scale for clean/dirty appearance with 1 = very clean completely healed with new callus; 2 = clean with new callus but some necrotic tissue; 3 = intermediate appearance, slightly recessed areas but no watersoaking on green wood; 4 = dirty appearance, cankers open and brown; 5 = very dirty, cankers recessed open and brown. Classes 1-3 were considered resistant and 4-5 susceptible. The variable CDA refers to this rating scale.

Leafspot severity for the whole tree was assessed in September 1990 and August 1991. The optimum time was considered a balance of maximum bacterial spot progression and minimum defoliation due to rust (*Tranzschelia discolor* (Fokl.) Tranz and Litv.). The rating scale was a modification of the 0 to 5 scale of Werner et al. (1986) with 0 = no symptoms; 1 = 0% to 1% of leaves with lesions; 2 = 1% to 5%; 3 = 5% to 15%; 4 = 15% to 40%; and 5 = over 40% of leaves with lesions. The variable LSPOT refers to this rating scale.

Statistical Analysis

Analysis of variance for individual years was performed with Model 3 (Table 4.2) and combined years analysis with Models 5 and 7 (Table 4.3) of the Least-Squares Maximum Likelihood Mixed and Weighted (LSMLMW) computer

program (Harvey, 1990). This program allows analysis of large sets of random effects by use of absorption techniques and so is well-suited for genetic variance component estimation (Koonce, 1990). Heterogeneity of variance was detected in plots of residual against predicted values; thus the variables LS12 and CANKA were transformed by natural log plus one and natural log, respectively (Schlotzhauer and Littell, 1987). Transformed values were used for analysis of variance, mean comparison, and heritabilities, but untransformed means are presented for clarity (Gomez and Gomez, 1984).

State and year were considered as fixed effects and genotype as a random effect. Variances were estimated by equating the mean squares to their mathematical expectations and solving. Variances were estimated for genotype within state, i.e., G(S), and residual error effects for single year analyses and for the additional terms of tree(G, S) and G(S)xY for the combined year analysis. The residual error variance (σ^2_{e2}) in Table 4.3 is a combination of the true error variance (σ^2_{e1}) and the variance due to T(S,G)xY interaction ($\sigma^2_{T(GS)xY}$); these terms cannot be separated in this analysis. The residual error mean square was used as the denominator in the F test for T(G,S), which is a conservative test. The variance component $\sigma^2_{T(GS)}$ is underestimated to the extent that $\sigma^2_{T(GS)xY}$ is greater than zero, and so the broad sense heritabilities estimated in the combined years analysis are biased upward.

It should be noted that Harvey's Model 7 EMS differ from the balanced case expectations of Schultz (1955) with the inclusion of $\sigma^2_{G(S)xY}$ in the G(S) line. This results in a more conservative estimate of broad sense heritability whenever the estimate of $\sigma^2_{G(S)xY}$ is positive. Variance estimates from Harvey Model 7 corresponded closely to estimates obtained from the SAS VARCOMP procedure using method = type 1 option (SAS, 1987), which are reported as satisfactory for among group variance estimation except when data are severely unbalanced (Littell and McCutchen, 1987).

Broad sense heritabilities are clonal repeatabilities (Burton and DeVane, 1953; Falconer, 1989) and were calculated for LS12, LSPOT, CANKA and CDA in 1990, 1991, and for combined years. Standard errors were calculated by Harvey's LSMLMW program (Swiger et al., 1964).

Results and Discussion

It is hard to imagine 2 years with more contrasting conditions for bacterial spot development than the 2 years during which this study was conducted. The average rainfall for Gainesville from Mar. to May is 230 mm (USDA, 1941), but in 1990 only 180 mm fell and 1991 was wetter than average with 642 mm. The average number of spots per inoculated leaf and leafspot ratings more than doubled, and the average length of inoculated cankers also increased significantly

from 1990 to 1991 (Table 4.4). However, the CDA rating did not change, indicating that the appearance of the inoculated cankers may be a more useful measure of stem canker resistance than the length of the canker. The correlation of genotype means between 1990 and 1991 was low for the leaf traits but moderately high for CANKA and CDA, indicating that although the length of cankers increased in 1991, the genotypes that produced longer cankers in 1990 also tended to produce longer cankers in 1991. The higher correlation between years for LS12 than for LSPOT shows the advantage of using an artificial inoculation system, i.e., there was a greater association between genotype means for the inoculated leaf count of spot number (LS12) than for the whole tree rating of infection severity (LSPOT).

States differed significantly in levels of bacterial spot on leaves and stems (Table 4.5) with Georgia consistently ranking as the state with most resistant germplasm and California as the state with most susceptible germplasm. Florida germplasm is selected for *X. campestris* pv. *pruni* resistance on the basis of stem canker ratings each winter (W.B. Sherman, pers. comm.) which explains the relatively lower levels of stem canker compared to leaf spot in this population. The trend of higher levels of resistance from the Georgia, Florida, and Queensland programs, where *X. campestris* pv. *pruni* is a problem, compared to California where no selection pressure for resistance is necessary, is not surprising

and concurs with findings of Topp et al. (1989). The genotypes sampled to represent each breeding program greatly affect the outcome of this result. Georgia, Florida, and California were sampled adequately but quarantine restrictions prevented a thorough sampling of clones from Queensland and only clones currently available in the USA were tested. The small number and restricted sampling technique for clones representing the Queensland germplasm are caveats that go with these estimates.

The effects of genotype within state effect were highly significant in 1990 and 1991 and for the combined analysis (Tables 4.6 and 4.7) indicating there was genetic variability for all traits. The error MS in Table 4.6 is a measure of differences among ramets within the one genotype and, as all ramets are genetically identical, this is the non-genetic or environmental variance. Partitioning of between and within genotype variability allows estimation of the total genetic variance and broad sense heritability.

State x year interaction was not significant for the 0-5 rating of leafspot severity and the average number of lesions per inoculated leaf and while significant for CANKA and CDA it was not a large component of variation compared to either the state or year effects (Table 4.7). This indicates that the ranking of states did not alter markedly from 1990 to 1991. State and year are

fixed effects and so their interactions also would be fixed and not contribute variance in the estimation of heritability.

The interactions of genotype(state) x year involve both random and fixed effects and contributes variance to heritability estimation (Table 4.7). This is the genotype x environment interaction term in this model. It was highly significant for the leafspot traits LS12 and LSPOT (F = 3.52 and 6.65, respectively) but less significant for CANKA and CDA (F = 1.78 and 2.58, respectively). This indicates that the ranking of genotypes within each state altered markedly from 1990 to 1991 for the leafspot traits but to a smaller extent for the stem canker traits. The consequences of this interaction are seen in the broad sense heritability (H_B) estimates (Table 4.8). Leafspot traits had H_B estimates ranging from 0.4 to 0.6 calculated for individual years but dropped to 0.206 and 0.331 for the combined analysis. The single year H_B estimates have the G(S) x Y interaction term confounded with the variance of G(S) in the numerator whereas the G(S) x Y term is separated in the combined years analysis. When there is smaller G(S) x Y interaction, as is the case for stem canker traits, then there is less change in H_B when year is included in the model. However when the G(S) x Y term is large, as occurred for the leafspot traits, then the H_B from the combined years analysis is reduced.

LS12 is an objective measure of the number of lesions per inoculated leaf whereas LSPOT is a subjective measure of leaf spot severity on the whole tree after natural infection using a 0-5 scale. LSPOT H_B increased from 0.4 in 1990 to 0.6 in 1991 (Table 4.8). The wet 1991 season resulted in fewer escapes (genotypes not showing symptoms due to inadequate pathogen pressure) and increased differences between genotypes without much change of the within-genotype-variability. This can be seen by comparing the MS for G(S) and error for LSPOT in Table 4.6. A higher H_B was obtained for the trait measuring natural infection in the year with the best conditions for disease development. In contrast the artificial inoculation trait LS12 showed less change in H_B over the 2 years and the ratio of MS for G(S) and error were similar across years.

The average length (mm) of the three inoculated cankers measured in CANKA during the summer also was measured at the end of the 1990 growing season. H_B for this trait in 1990 was 0.658 ± 0.057 indicating that this is a superior trait upon which to base selection for stem canker resistance. It is also a more convenient time to measure stem cankers when leaves have fallen and fruit breeders have begun their winter hibernation.

High levels of leafspot and stem canker resistance were identified in several genotypes (Table 4.9). An overall rating of leafspot (Table 4.10) and stem canker (Table 4.11) resistance was calculated using a selection index. Leafspot selection

index was constructed by calculating the standardized LS12 and LSPOT ratings for 1990 and 1991 (i.e., expressing each value as a deviation from the population mean and dividing by the standard deviation), weighting these by their respective H_B estimates and then summing the four values. H_B is a measure of clonal repeatability and so measures the precision of each trait - year combination. Stem canker selection index was calculated in the same manner, but using CANKA and CDA traits. The most resistant genotypes were almost all from Georgia and include several cultivars such as 'Segundo' and 'Robusto' which are reported to have good fruit quality (Tehrani et al., 1991; Brooks and Olmo, 1982). The selections coded 306, 317 and 324 were common to both tables and so have good levels of resistance to bacterial leafspot and stem canker. Selections such as 310 and 319 were resistant to leafspotting but susceptible to canker development (Table 4.10). Conversely, selections 301 and 311 were among the most stem canker resistant genotypes but rated poorly for LS12 (Table 4.11).

Summary and Conclusions

Germplasm from the four breeding programs differed in levels of bacterial spot resistance with high levels of leafspot and stemcanker resistance identified particularly in genotypes from Georgia. The wet season in 1991 was ideal for bacterial spot development and contrasted with the dry season of 1990. There

were significant genotype x year interactions for leaf spot resistance and stem canker resistance, but the stem canker traits were more stable over years which resulted in less change in H_B for the combined two-year analysis. The H_B estimates indicated that, while artificial inoculation of leaves will allow more gain in dry years than relying on rating of natural infection, it is similar to selection based on whole tree ratings in an ideal year for disease development. There was an interesting trend of increasing precision for leafspot traits in the wet year, but for decreasing precision for stem canker traits. This indicates that the environment which favored measurement of leaf traits adversely affected measurement of stem traits.

Table 4.1. Names and selection numbers of genotypes sampled from breeding programs at California, Florida, Georgia and Queensland.

California[z]		Florida[z]		Georgia[z]		Queensland[z]	
Code	Clone name/ number	Code	Clone name/ number	Code	Clone name/ number	Code	Clone name/ number
101	K10-1	201	FL3-5	301	BY68-1119	401	Beauty
102	K42-26	202	FL30-37	302	BY8158-50	409	Kelsey
103	K43-49	203	FL30-43	303	BY8304-17	410	Mariposa
104	K141-15	204	FL30-47	304	BY8306-17	412	Purple
105	K242-45	205	FL31-75	305	BY8313-14	416	Simka
106	Angelino	206	FL34-12	306	BY8334-19	417	Toka
107	Blackamber	207	FL35-5	307	BY8334-45	420	Wilson
108	Casselman	209	FL85-1	308	BY8335-71		
109	Friar	210	FL85-2	309	BY8336-130		
110	Laroda	211	FL85-3	310	BY8339-48		
111	Queenrosa	212	FL86-1	311	BY8401-266		
112	Santarosa	213	FL87-2	312	BY8401-869		
113	Wickson	214	Gulfgold	313	BY8401-936		
114	Early Burmosa	215	Gulfruby	314	BY8402-331		
116	Redbeaut	216	Wade	315	BY8402-373		
				316	BY8402-390		
				317	Bruce		
				318	Byrongold		

319	FLA1-1
320	BYM380
321	Methley
322	Robusto
324	Segundo
326	BY8402-428
327	Rubysweet
328	BY8402-428
331	BY69-924
332	BY82E2065
333	BY8351-16

[z]Breeding programs of CA = USDA, Fresno, California; FL = University of Florida, Gainesville, Florida; GA = USDA, Byron, Georgia; QL = Queensland Dept. Primary Industries, Applethorpe, Queensland, Australia.

Table 4.2. Expectations of mean squares (EMS) and coefficients for analysis of average leafspot number per inoculated leaf (LS12), leafspot rating (LSPOT), average length (mm) of inoculated cankers (CANKA) and canker appearance rating (CDA) for individual years 1990 and 1991.

Source	df 1990	df 1991	EMS
State	3	3	$\sigma_e^2 + k\,\sigma_{G(S)}^2 + k\,\Theta_S^2$
Genotype (S)	56	62	$\sigma_e^2 + k_1\,\sigma_{G(S)}^2$
Error	148	217	σ_e^2
	Coefficients		
k_1	3.4208	4.2617	

Table 4.3. Expectations of mean squares (EMS) and coefficients for combined years analysis of variance of average number of lesions per inoculated leaf, leafspot severity rating, average length of inoculated cankers and canker appearance rating.

Source	df	EMS
State	3	$\sigma^2_{e1} + k\,\sigma^2_{T(GS)} + k\,\sigma^2_{G(S)} + k\,\Theta^2_S$
Genotype (S)	62	$\sigma^2_{e1} + k_3\,\sigma^2_{T(GS)} + k_4\,\sigma^2_{G(S)}$
Tree(GS)	222	$\sigma^2_{e1} + k_2\,\sigma^2_{T(GS)}$
Year	1	$\sigma^2_{e2} + k\,\sigma^2_{G(S)xY} + k\,\Theta^2_Y$
S x Y	3	$\sigma^2_{e2} + k\,\sigma^2_{G(S)xY} + k\,\Theta^2_{SxY}$
G(S) x Y	56	$\sigma^2_{e2} + k_1\,\sigma^2_{G(S)xY}$
Residual	143	σ^2_{e2}
	Coefficients	
k_1	3.7378	
k_2	1.6832	
k_3	1.7635	
k_4	7.3515	

where $\sigma^2_{e2} = \sigma^2_{e1} + \sigma^2_{T(GS)xY}$

Table 4.4. Least squares means (± standard error) for 1990 and 1991 for the average number of lesions per inoculated leaf (LS12), leafspot severity rating (LSPOT), average length (mm) of inoculated cankers (CANKA) and canker appearance rating (CDA) and correlations between genotypic means for 1990 and 1991.

Trait	1990	1991	r[z]
LS12 (lesions/leaf)	16.10 ±3.49	47.69 ±3.28	0.44**
LSPOT (0-5)	0.64 ±0.14	4.25 ±0.13	0.04[NS]
CANKA (mm)	10.75 ±0.79	21.27 ±0.75	0.59**
CDA (1-5)	3.96 ±0.16	4.15 ±0.15	0.65**
No. Observations	208	283	60

[NS], *,** Nonsignificant or significant at P ≤0.05 and 0.01, respectively.

[z]Correlations are on transformed values of LS12 and CANKA.

Table 4.5. Least squares means for measuring average number of lesions per inoculated leaf (LS12), leafspot severity rating (LSPOT), average length (mm) of inoculated cankers (CANKA) and canker appearance rating (CDA) for plum clones from breeding programs at California, Florida, Georgia and Queensland.

		Trait means[z]			
State	Number of observations	LS12 (lesions/ leaf)	LSPOT (0-5)	CANKA (mm)	CDA (1-5)
California	95	42.5 a[y]	2.7 a	21.1 a	4.6 a
Florida	111	36.6 ab	2.7 a	13.4 b	4.3 ab
Georgia	224	24.2 b	2.1 b	13.3 b	3.4 b
Queensland	61	24.3 b	2.3 ab	16.1 b	3.9 ab

[z]Each mean is of 2 years, 2 to 6 ramets/genotype, and 7 to 29 genotypes/state.

[y]Mean separation within columns by multiple t-test at $P \leq 0.05$.

Table 4.6. Individual year analysis of variance for number of lesions per inoculated leaf (LS12), leafspot severity rating (LSPOT), average length (mm) of inoculated cankers (CANKA) and canker appearance rating (CDA).

Trait	Source	1990 MS	1990 F	1991 MS	1991 F
LS12[z]	State	4.65	2.87*	7.65	5.02**
	Genotype (S)	1.62	4.35**	1.53	5.64**
	Error	0.37		0.27	
LSPOT	State	7.01	4.78**	10.28	2.50[NS]
	Genotype (S)	1.47	3.20**	4.11	7.15**
	Error	0.46		0.58	
CANKA[z]	State	2.02	4.36**	4.57	12.95**
	Genotype (S)	0.46	4.64**	0.35	2.99*
	Error	0.10		0.12	
CDA	State	29.37	5.59**	12.12	3.62*
	Genotype (S)	5.26	5.86**	3.35	3.74**
	Error	0.90		0.90	

[NS],*,** Nonsignificant or significant at P $\leq$0.05 and 0.01, respectively.

[z]Transformed as ln (LS12 + 1) and ln (CANKA).

Table 4.7. Combined years analysis of variance for average number of lesions per inoculated leaf (LS12), leafspot severity rating (LSPOT), average length (mm) of inoculated cankers (CANKA) and canker appearance rating (CDA).

	LS12[z]		LSPOT		CANKA[z]		CDA	
Source	MS	F	MS	F	MS	F	MS	F
State	11.33	5.31**	12.19	4.28**	4.81	7.62**	38.18	6.07**
Genotype (S)	2.13	6.26**	2.85	4.83**	0.63	5.25**	6.29	6.48**
Tree(GS)	0.34	1.26[NS]	0.59	1.37*	0.12	1.33*	0.97	1.24[NS]
Year	105.30	110.84**	1070.09	374.16**	41.47	259.19**	2.94	1.46[NS]
S x Y	1.33	1.40[NS]	3.76	1.31[NS]	0.82	5.13**	7.28	3.62*
G(S) x Y	0.95	3.52**	2.86	6.65**	0.16	1.78*	2.01	2.58**
Residual	0.27		0.43		0.09		0.78	

[NS],*,** Nonsignificant or significant at P ≤0.05 and 0.01, respectively.

Table 4.8. Broad-sense heritability estimates (± SE) for 1990, 1991 and combined years analysis for average number of spots per inoculated leaf (LS12), leafspot severity rating (LSPOT), average length (mm) of inoculated stem cankers (CANKA) and canker appearance rating (CDA).

	Broad-sense heritability[z]		
Trait	1990	1991	Combined
LS12[y]	0.499 ± 0.071	0.526 ± 0.061	0.331 ± 0.053
LSPOT	0.396 ± 0.076	0.597 ± 0.057	0.206 ± 0.047
CANKA[y]	0.520 ± 0.070	0.322 ± 0.066	0.350 ± 0.054
CDA	0.593 ± 0.064	0.395 ± 0.065	0.371 ± 0.054

[z]Single tree broad sense heritabilities (H_B) estimated as:

For single years $H_B = \frac{\sigma^2_{(G)S}}{\sigma^2_{G(S)} + \sigma^2_e}$.

For combined years $H_B = \frac{\sigma^2_{G(S)}}{\sigma^2_{G(S)} + \sigma^2_{T(GS)} + \sigma^2_{G(S)xY} + \sigma^2_e}$.

[y]Values were transformed as ln (LS12 + 1) and ln (CANKA).

Table 4.9. Genotype means (average of 4 ramets/genotype) for 1990 and 1991 for the traits average number of lesions per inoculated leaf (LS12), leafspot severity rating (LSPOT), average length of inoculated cankers (CANKA), and canker appearance rating (CDA).

	1990				1991			
Genotype[z]	LS12 (lesions/leaf)	LSPOT (0-5)	CANKA (mm)	CDA (1-5)	LS12 (lesions/leaf)	LSPOT (0-5)	CANKA (mm)	CDA (1-5)
101	23.9	2.5	28.3	2.3	108.4	5.0	36.0	5.0
102	4.0	0.0	4.3	3.8	45.0	4.8	34.6	4.3
103	.	0.3	.	1.5	55.3	3.5	49.3	5.0
104	19.0	0.0	16.7	5.0	19.8	4.3	29.6	5.0
105	10.5	1.0	15.0	5.0	47.8	4.0	37.9	5.0
106	7.2	1.0	13.1	3.0	62.8	5.0	29.2	5.0
107	15.7	1.5	15.7	4.2	97.2	5.0	35.7	5.0
108	9.7	0.3	9.1	5.0	41.2	4.7	20.3	5.0
109	19.0	1.5	12.0	4.0	160.8	4.8	38.0	5.0
110	70.0	1.0	13.0	5.0	22.3	5.0	28.0	5.0
111	40.5	1.8	7.0	2.3	120.9	4.3	23.0	4.8
112	12.0	0.8	9.8	4.3	56.6	5.0	22.3	4.5
113	12.0	0.0	6.8	3.0	35.4	4.0	17.2	2.2
114	37.1	0.2	13.2	3.3	39.1	4.8	30.7	5.0
116	22.3	0.6	13.7	4.0	40.0	4.3	21.4	4.7
201	7.0	1.0	8.9	5.0	11.2	4.3	12.0	4.0
202	10.2	0.4	12.3	3.5	52.3	4.4	15.9	5.0
203	26.4	1.0	8.9	4.5	82.9	2.6	12.1	3.8
204	15.5	0.0	9.5	5.0	24.5	5.0	15.5	4.5

Table 4.9.--Continued.

Genotype[z]	1990				1991			
	LS12 (lesions/leaf)	LSPOT (0-5)	CANKA (mm)	CDA (1-5)	LS12 (lesions/leaf)	LSPOT (0-5)	CANKA (mm)	CDA (1-5)
205	15.0	2.3	13.0	4.0	68.3	3.7	15.0	3.3
206	9.3	0.5	8.8	5.0	75.0	5.0	15.8	4.5
207	13.8	0.7	10.7	3.7	49.3	4.0	17.0	4.3
209	7.0	1.2	10.3	4.5	34.6	3.6	18.1	3.8
210	22.1	1.5	10.0	4.0	67.4	5.0	19.3	3.8
211	7.3	1.3	11.8	3.7	76.3	4.7	16.0	5.0
212	12.8	1.8	10.3	3.0	21.4	4.5	19.4	4.3
213	16.8	1.4	7.3	3.4	101.0	5.0	13.3	3.8
214	12.1	1.2	7.1	3.0	33.2	4.2	11.9	3.6
215	10.9	1.8	14.1	2.5	118.0	5.0	26.7	5.0
216	21.5	0.0	4.3	2.3	28.8	4.5	17.2	4.0
301	61.3	0.0	4.3	4.3	20.5	3.7	9.1	2.3
302	9.1	0.7	5.7	3.8	18.6	4.0	16.2	2.4
303	13.1	0.4	5.6	4.5	32.8	3.7	18.4	3.7
304	15.0	0.3	8.4	3.0	63.8	5.0	18.4	5.0
305	10.5	0.4	15.8	3.7	83.8	4.4	23.9	5.0
306	7.9	0.0	3.7	5.0	32.5	3.8	15.2	1.8
307	9.4	0.2	6.6	4.2	36.0	4.8	16.7	3.8
308	12.0	0.5	13.3	5.0	20.0	5.0	19.2	5.0
309	27.3	0.0	7.2	3.0	34.8	4.5	16.2	2.0
310	5.3	0.2	7.0	4.5	13.8	4.2	20.1	5.0
311	5.8	0.5	4.4	2.5	57.1	5.0	17.5	3.3

Table 4.9.--Continued.

Genotype[z]	1990				1991			
	LS12 (lesions/leaf)	LSPOT (0-5)	CANKA (mm)	CDA (1-5)	LS12 (lesions/leaf)	LSPOT (0-5)	CANKA (mm)	CDA (1-5)
312	14.0	0.0	7.4	3.7	32.4	4.8	14.0	2.5
313	19.0	0.7	4.6	3.6	49.0	5.0	18.2	2.8
314	5.4	0.0	6.2	5.0	24.5	4.6	16.6	4.8
315	18.5	0.0	7.4	2.3	21.7	5.0	21.9	4.7
316	14.3	0.0	6.9	3.0	34.4	5.0	13.9	4.0
317	2.9	.	4.7	2.5	14.7	1.2	9.8	3.2
318	53.6	0.0	17.1	3.7	67.9	2.3	22.9	4.0
319	2.1	1.7	11.6	4.7	14.9	2.2	22.0	5.0
320	20.3	0.8	11.4	3.7	60.1	5.0	34.3	5.0
321	18.0	0.4	16.0	4.3	50.6	5.0	27.2	4.5
322	3.4	0.6	6.7	5.0	9.0	1.0	19.6	3.8
324	1.9	1.4	5.3	2.6	9.8	1.2	16.5	3.0
326	13.0	2.5	20.8	3.0	75.8	5.0	34.2	5.0
327	9.0	0.0	7.3	3.3	31.8	4.5	9.3	2.5
328	5.0	0.0	5.4	4.3	36.0	2.6	14.7	2.6
331	0.5	0.3	7.0	3.7	11.0	4.7	14.6	2.3
332	1.8	0.0	10.1	3.8	9.9	4.0	16.6	3.8
333	1.5	0.0	4.6	4.2	46.5	4.0	17.1	4.8
401	11.4	0.3	15.1	3.5	24.2	4.0	27.2	5.0
409	33.8	1.2	13.8	3.0	51.1	4.8	22.3	5.0

Table 4.9.--Continued.

Genotype[z]	1990				1991			
	LS12 (lesions/leaf)	LSPOT (0-5)	CANKA (mm)	CDA (1-5)	LS12 (lesions/leaf)	LSPOT (0-5)	CANKA (mm)	CDA (1-5)
410	10.4	0.0	6.1	3.8	15.5	4.2	17.5	3.8
412	12.8	0.4	7.6	3.4	38.1	4.3	22.7	4.4
416	8.0	0.0	8.3	5.0	33.3	4.0	17.6	2.8
417	.	3.5	8.0	4.5	57.8	4.3	24.6	4.3
420	10.9	0.4	14.7	4.0	25.3	4.3	21.0	3.7

[z]See Table 4.1 for genotype code names/numbers and state of origin.

Table 4.10. Plum clones with highest leaf spot resistance as measured by a selection index (SI) of average number of lesions per inoculated leaf (LS12) and leafspot severity rating on a 0 to 5 scale (LSPOT).

Clone			Traits[z]				
Code	Designation	State	LS12 (lesion/ leaf)	LSPOT (0-5)	CANKA (mm)	CDA (1-5)	SI[y]
322	Robusto	GA	6.2	0.8	13.1	4.4	-2.42
317	Bruce	GA	8.8	1.2	7.3	2.9	-2.23
324	Segundo	GA	5.9	1.3	10.9	2.8	-2.05
328	BY8402-428	GA	21.7	1.8	10.0	3.4	-1.42
332	BY82E2065	GA	6.7	2.2	13.9	3.8	-1.41
319	FLA1-1	GA	8.5	1.9	16.8	4.8	-1.39
306	BY8334-19	GA	20.2	2.1	9.4	2.8	-1.07
310	BY8339-48	GA	11.4	2.2	15.2	4.8	-0.85
333	BY8351-16	GA	26.0	2.2	10.9	4.5	-0.82
303	BY8304-17	GA	22.9	2.2	12.0	4.0	-0.79

[z]Genotype means (n = 6 to 12) over 1990 and 1991.

[y]Selection Index is the sum of standardized LS12 and LSPOT values in 1990 and 1991 weighted by their respective repeatabilities (broad sense heritabilities).

Table 4.11. Plum clones with highest stem canker resistance as measured by a selection index (SI) of average length (mm) of inoculated cankers (CANKA), and canker appearance rating on a 1 to 5 scale (CDA).

Clone			Traits[z]				
Code	Designation	State	CANKA (mm)	CDA (1-5)	LS12 (lesions/ leaf)	LSPOT (0-5)	SI[y]
317	Bruce	GA	7.3	2.9	8.8	1.2	-1.52
113	Wickson	CA	12.0	2.6	23.7	2.0	-1.32
309	BY8336-130	GA	11.7	2.5	31.0	3.0	-1.32
324	Segundo	GA	10.9	2.8	5.9	1.3	-1.32
327	Rubysweet	GA	8.5	2.9	22.7	2.3	-1.23
301	BY68-1119	GA	6.7	3.3	40.9	1.8	-1.19
311	BY8401-266	GA	11.9	2.9	31.4	2.8	-1.18
306	BY8334-19	GA	9.4	2.8	20.2	2.1	-1.09
216	Wade	FL	10.8	3.3	26.3	2.6	-1.04
331	BY69-924	GA	10.8	3.0	6.8	2.5	-1.03

[z]Genotype means (n = 6 to 12) over 1990 and 1991.

[y]Selection Index is the sum of standardized CANKA and CDA values in 1990 and 1991 weighted by their respective repeatabilities (broad sense heritabilities).

CHAPTER 5

HERITABILITY OF LEAF AND STEM RESISTANCE TO *XANTHOMONAS CAMPESTRIS* PV. *PRUNI* IN A DIVERSE POPULATION OF JAPANESE PLUM

Introduction

Narrow sense heritability is the relative part of total variance associated with additive genetic variance and is useful in predicting population changes in response to selection (Falconer, 1989). Genetic gain in response to selection depends on the selection differential and the heritability, with heritability expressed on the same unit basis as the type of selection (Hansche, 1983; Hanson, 1963). Prediction of response when more than one trait is selected requires knowledge of the appropriate variances, genetic correlations among the traits and the heritability estimates. High phenotypic correlations are not necessarily indicative of high genetic correlations because the phenotypic correlation is a weighted sum of both environmental and genetic correlations (Becker, 1984; Falconer, 1989). *Xanthomonas campestris* pv. *pruni* (Smith) Dye on Japanese-type plum (*Prunus salicina* L.) produces symptoms of leaf spots, stem cankers and star-cracks on fruit (Heaton, 1983). Resistance is required in all three organs due to

reduced photosynthetic area from leaf infection, stem breaking and overwintering of bacteria in stem cankers (Moffet, 1973), and reduced commercial value of blemished fruit. Heritabilities of these traits and correlations among them have not been reported for plum. Popenoe (1959) reported there was no relation between susceptibility to stem cankers and susceptibly to fruit and leaf spots but did not publish data. Werner et al. (1986) reported significant phenotypic correlations of 0.3 to 0.5 between leaf infection severity and fruit infection severity on a population of peach and nectarine. Layne (1966) concluded that leaf and fruit resistance in apricot were under separate genetic control due to genotypes occurring in all four classes of susceptible/resistant reactions to the two traits (i.e., leaf resistant/fruit susceptible, leaf resistant/fruit resistant, leaf susceptible/fruit susceptible, and leaf susceptible/fruit resistant). The purpose of the present study was to estimate genetic parameters for resistance to bacterial spot for both leaf and stem in order to predict response to selection.

Materials and Methods

Plant Material and Experimental Design

Open pollinated seeds were collected from breeding programs at the United States Department of Agriculture (USDA), Fresno, California; University of Florida, Gainesville, Florida; USDA, Byron, Georgia; and the Queensland

Department of Primary Industries, Queensland, Australia. Japanese plums are mostly self-infertile, and so the seed from each parent tree were considered members of a half-sib family (Weinberger, 1975). Seed were stratified, germinated and field planted in Gainesville, Florida between Apr. and Aug. 1989 to give a total population of 1555 seedlings from 84 families and four states. Half-sib families were planted in a seedling nursery (Sherman and Lyrene, 1983) in a completely random design with an average of 4.6 trees per plot, 4 plots per family and 21 families per state. Parent genotypes were clonally propagated on peach seedling rootstock and planted in winter 1989 adjacent to the seedling nursery but in separate, randomized, single tree plots with an average of four ramets per parent genotype. Tree spacings were 90 cm between rows and 30 cm between trees. Bacterial spot susceptible 'Gulfruby' trees were planted along alleyways every 4 m to ensure even inoculum load. Supplementary irrigation was by overhead sprinkler; bactericide sprays were not applied. Trees were headed back to about 15 cm above ground level in winter to force vigorous new shoots the following spring. The severe pruning removed infected branches and prevented uneven distribution of inoculum the following season.

Inoculation and Rating Methods

Leaves were inoculated between 6:00 and 8:00 AM in June 1990 and May 1991. Stems were inoculated on the same day as leaves but between 8:00 AM and 9:00 PM. One actively growing branch per tree was tagged and the top 10 leaves were immersed in 2.5 x 10^8 cfu per ml *X. campestris* pv. *pruni* inoculum and agitated for 5 seconds until leaf surfaces were fully wetted. The same branch was injected at three sites within the top 15 cm of stem using a 26 gauge needle and syringe and 2.5 x 10^8 cfu per ml inoculum. The number of lesions on the two leaves with highest incidence of disease were counted 14 days after inoculation. The average number of spots on these two leaves is the variable LS12.

Length of the three stem cankers was measured 6 weeks after inoculation. The average length (mm) of these three cankers is the variable CANKA. Cankers were rated at this time on a 1 to 5 scale for clean/dirty appearance with 1 = very clean completely healed with new callus; 2 = clean with new callus but some necrotic tissue; 3 = intermediate appearance, slightly recessed areas but not watersoaking on green wood; 4 = dirty appearance, cankers open and brown; 5 = very dirty, cankers recessed open and brown. Classes 1-3 were considered resistant and 4-5 susceptible. The variable CDA refers to this rating scale.

Leafspot severity for the whole tree was assessed in Sept. 1990 and Aug. 1991. The optimum time was considered a balance of maximum bacterial spot

progression and minimum defoliation due to rust (*Tranzschelia discolor*). The rating scale was a modification of the 0 to 5 scale of Werner et al. (1986) with 0 = no symptoms; 1 = 0% to 1% of leaves with lesions; 2 = 1% to 5%; 3 = 5% to 15%; 4 = 15% to 40% of leaves with lesions. The variable LSPOT refers to this rating scale.

Statistical Analysis

Analysis of variance for individual years (Table 5.1) was performed with Model 5 of the Least-Squares Maximum Likelihood Mixed and Weighted (LSMLMW) computer program (Harvey, 1990). This program allows analysis of large sets of random effects by use of absorption techniques and so is well suited for genetic variance component estimation (Koonce, 1990). The combined years analysis (Table 5.2) was performed using successive runs of Harvey's Models 1, 5 and 7. Variance components were estimated by equating the mean squares to their mathematical expectations. State, year and state x year were considered fixed effects and all other effects were considered random. Japanese plums are mostly self-infertile and so individuals within an open pollinated family were considered half-sibs (Weinberger, 1975). Narrow sense heritabilities (h^2) with individual trees as the selection unit were estimated as four times the family within state variance ($\sigma^2_{F(S)}$) divided by the sum of all variance components (Falconer,

1989). Standard errors were calculated as described by Falconer (1989). Phenotypic, genetic and environmental correlations and standard errors were calculated using Harvey's Model 5 (Harvey, 1990).

The residual error variance (σ^2_{e2}) in Table 5.2 is the sum of the true error variance (σ^2_{e1}) and the variance due to T(PFS)xY interaction ($\sigma^2_{T(PFS)xY}$); these terms are confounded in this analysis. The residual error mean square was used as the denominator in the F test for T(PFS), which is a conservative test. The variance component $\sigma^2_{T(PFS)}$ was estimated using the residual error variance (σ^2_{e2}). This gives an underestimate of $\sigma^2_{T(PFS)}$ to the extent that $\sigma^2_{T(PFS)xY}$ is greater than zero, and so the narrow sense heritabilities estimated for combined years by half-sib family variance analysis are biased upward.

Individual offspring values were regressed on female parent means for 1990 and 1991, and the regression coefficients (and standard errors) multiplied by two to obtain heritabilities (Bohren et al., 1961). Parent and offspring values were adjusted for year effects, by adding the difference of the 1991 and 1990 year means to the 1990 values, prior to regressing offspring on parents for the combined year estimates of heritability.

Results and Discussion

Average levels of bacterial spot varied between states (Tables 5.3 and 5.4) and years (Table 5.5) for all traits. California germplasm had highest levels of bacterial spot for stem cankers in 1990 and for all traits in 1991 (Table 5.3). Georgia germplasm had lowest levels of leaf spots and stem cankers in 1990 and 1991. Bacterial spot is not a problem in the dry climate of California and so there is no selection for resistance, but in the southeastern United States bacterial spot is endemic and selection for resistance is a high priority. Florida germplasm had high levels of leaf spot (as measured by LS12 and LSPOT) but was not significantly different from the Georgia material in length of inoculated cankers (CANKA). This is probably due to the selection methods at Florida where trees are culled for *X. campestris* pv. *pruni* susceptibility in winter by visually rating stem canker severity. The stem canker appearance rating (CDA) indicates Florida germplasm is not as resistant as Georgia germplasm (Table 5.3).

There were significant differences among families within states for all traits in the combined years analysis (Table 5.5) and for all but LS12 in the individual year analysis (Table 5.4). The family within state component of variance is the observational component which is equal to the covariance among the half-sib family members, and so has a genetic expectation of one-fourth the additive variance (Falconer, 1989). This term is important in determining narrow sense heritability. For the combined years analysis the F(S) variance accounted for only

3.2% of the total variance for LS12 but 11.0% for CANKA (Table 5.6). The F(S)xY components of variance were low for all traits accounting for a maximum of 4.2% for CDA. This contrasts with the analysis of parent genotypes in Chapter 4 where genotype x year interactions were relatively large for LSPOT and LS12. The largest sources of variation were due to tree within plot-family-state groups and to the residual error (Table 5.6). Plums are highly heterozygous and outcrossing (Weinberger, 1979), and so it is not surprising that there was large variation among seedling trees within a plot.

The variance among half-sib families within each state ($\sigma^2_{F(S)}$) is a pooled estimate across the four states. Heritabilities calculated using this as one-fourth of the additive genetic variance are appropriate for selection within any one of the breeding programs. On this basis stem canker length and stem canker appearance are more heritable than leaf spot number and leaf spot severity (Table 5.7). The estimates for LSPOT, CANKA, and CDA are low to moderate when compared to estimates for other fruit tree traits (Hansche, 1983). There are few heritability estimates of bacterial diseases in fruit trees with which to compare our estimates. Bell et al. (1977) reported narrow sense heritability for fireblight resistance in pear of 0.52. Chang et al. (1991) reported a heritability from parent-offspring regression for canker resistance to the fungus *Leucostoma* in peach of 0.72.

Regression of individual offspring values on parent means was used to estimate heritability for 1990, 1991, and for a combined-years analysis (Table 5.8).

The combined-years heritability estimates are similar in value to the heritability estimates obtained from variance component analysis for LS12 and CDA, but are about doubled for LSPOT and CANKA. One of the assumptions in heritability estimation from parent offspring regression is no environmental correlation among relatives (Cockerham, 1963). Presence of positive genotype x environment interaction covariance and error covariance between parent and offspring will cause an upward bias in heritability estimates (Casler, 1982; Fernandez and Miller, 1985; Nguyen and Sleper, 1983). Falconer (1989) also discussed problems arising from using phenotypic covariance instead of genetic covariance in parent offspring regression. Error covariance in our experiment is expected to be near zero because parents and offspring were randomized independently of each other; i.e., they were not included in the same plots (Casler, 1982). However, our estimates may be biased upward by genotype x location and genotype x year interaction covariance. One technique to reduce this bias is to regress offspring means from one year on parental means from the other year (Casler, 1982). This gave estimates (± SE) of 0.043 (0.060), 0.444 (0.154), 0.952 (0.156) and 0.628 (0.168) for LS12, LSPOT, CANKA and CDA, respectively, for the combined year data. These values would still be biased by genotype x location interaction covariance.

If selection were practiced on a population which combined germplasm from all four breeding programs then the appropriate heritability would be calculated from a model that did not include state. The numerator for heritability

would then be four times the variance among families (rather than four times the variance among families within states). These estimates were calculated using Harvey's Model 4 and Model 6 for individual year and combined years, respectively (Table 5.9). Length of canker (0.795), canker appearance (0.645) and leaf spot severity (0.807) are moderate to highly heritable (Table 5.9). These estimates are higher than those based on the variance among families within states due to the increased variation among the total population of families. Germplasm exchange between the four breeding programs is possible, but is restricted by quarantine regulations (Australia versus USA) and by differences in selection objectives [i.e., low chilling requirement for Florida germplasm but high chilling for the other three states; and leaf scald (*Xylella fastidiosa* Wells et al.) resistance in Georgia and Florida but not in California or Queensland]. However, exchange of material is likely to increase in the future, and so these heritability estimates are of interest. The Queensland program would benefit from germplasm imports from other states because of an increase in heritability without a substantial loss in mean level of stem and leaf resistance (Table 5.3). The choice is not so clear for Georgia, which has the most resistant material and so could gain an increase in heritability at the expense of a reduced mean level of resistance.

Genetic correlations between leaf resistance traits and stem canker resistance traits were all positive and generally high (Table 5.10) indicating there are common genes for leaf and stem resistance and that selection for one trait will

result in improvement of the other. Phenotypic correlations were lower (0.1 to 0.2) than genetic correlations (0.5 to 0.9). The environmental correlations were close to zero and negative indicating that environmental conditions which favor development of stem cankers were unfavorable for producing large numbers of leaf spots.

Response to selection (R) per generation using phenotypic mass selection is defined as $R = i\, h^2\, \sigma_P$ where i is the selection intensity, h^2 is the narrow sense heritability on an individual tree basis, and σ_P is the phenotypic standard deviation (Falconer, 1989). Pooled heritability estimates from the half-sib family(state) analysis of variance (Table 5.7) for combined years and the parent offspring regression of opposite years were used to estimate response for leaf and stem traits (Table 5.11). A selection intensity of 20% for resistance to *X. campestris* pv. *pruni* would leave 1000 out of every 5000 seedlings for selection for other important traits and so is a realistic maximum for selection pressure. Stem cankers would be reduced by about 9 mm per generation and severity of leaf spots by 0.7 units. Positive correlated responses for all other bacterial spot traits would occur because of the positive genetic correlations (Table 5.10).

The high genetic correlation between LS12 and CANKA (0.872) and large difference in their heritabilities (0.128 and 0.438, respectively) makes indirect selection of leaf spot number via selection for canker length a possibility. The relative efficiency of indirect selection for character X (via direct selection for

character Y) compared to direct selection for character X is given by Falconer (1989) as

$$\frac{CR_x}{R_x} = \frac{r_G \sqrt{h_y^2}}{\sqrt{h_x^2}}$$

where CR_x is the correlated response of character X resulting from selection applied to character Y; R_x is the response of the character X if selection were applied directly to it; r_G is the genetic correlation between characters X and Y; and h_x^2 and h_y^2 are the narrow sense heritabilities of traits X and Y, respectively.

The relative efficiency of indirect selection for LS12 via direct selection for CANKA is

$$\frac{CR_x}{R_x} = \frac{0.872\ (\sqrt{0.438}\ )}{\sqrt{0.128}} = 1.6$$

Thus, it would be 1.6 times more efficient to select LS12 via CANKA rather than by direct selection for LS12.

Summary and Conclusions

Families within each breeding program differed in levels for two traits measuring leaf spot resistance (LS12 and LSPOT) and two traits measuring stem canker resistance (CANKA and CDA). Narrow sense heritability estimates were obtained by half-sib family analysis of variance and parent offspring regression with moderate to high (0.5 to 1.0) values for length (mm) of inoculated stem canker (CANKA) and moderate (0.3 to 0.5) values for leaf spot severity measured on a 0 to 5 scale. These traits will give greater gain in resistance than CDA or LS12. The problem with LSPOT is that it is less effective in years of low disease incidence because LSPOT relies on natural infection (see 1990 versus 1991 heritabilities for LSPOT in Tables 5.7 and 5.9). The high positive genetic correlation between CANKA and LS12 and the difference in their heritabilities indicate that selection solely for CANKA may be the most effective use of selection resources in improving both leaf spot and stem canker resistance. The parent offspring regression method (Table 5.8) generally gave higher heritability estimates than the half-sib family analysis of variance method (Table 5.7) but may be biased by non-genetic covariance components in the numerator of the regression coefficient. The moderate to high heritabilities obtained for CANKA by all methods indicate it is not greatly influenced by environment and may be under relatively simple genetic control. The high genetic correlations between leaf and stem traits indicates there are common genes controlling both traits.

Table 5.1. Expectations of mean squares (EMS), degrees of freedom (df) and coefficients for 1990 and 1991 analysis of variance of half sib families from four breeding programs (states).

	df		
State	1990	1991	EMS
State	3	3	$\sigma_e^2 + k\,\sigma_{P(FS)}^2 + k\,\sigma_{F(S)}^2 + k\,\theta_S^2$
Family (S)	75	80	$\sigma_e^2 + k_2\,\sigma_{P(FS)}^2 + k_3\,\sigma_{F(S)}^2$
Plot (FS)	242	276	$\sigma_e^2 + k_1\,\sigma_{P(FS)}^2$
Tree (PFS)	1125	1195	σ_e^2
	Coefficients		
k_1	4.4410	4.2356	
k_2	4.6207	4.5316	
k_3	17.7740	18.0605	

Table 5.2. Expectations of mean squares (EMS), degrees of freedom (df) and coefficients for combined years analysis of variance of half sib families from four breeding programs (states).

Source	df	EMS
State	3	$\sigma^2_{e1} + k\,\sigma^2_{T(PFS)} + k\,\sigma^2_{P(FS)} + k\,\sigma^2_{F(S)} + k\,\Theta^2_S$
Family (S)	80	$\sigma^2_{e1} + k_7\,\sigma^2_{T(PFS)} + k_8\,\sigma^2_{P(FS)} + k_9\,\sigma^2_{F(S)}$
Plot (FS)	280	$\sigma^2_{e1} + k_5\,\sigma^2_{T(PFS)} + k_6\,\sigma^2_{P(FS)}$
Tree (PFS)	1391	$\sigma^2_{e1} + k_4\,\sigma^2_{T(PFS)}$
Year	1	$\sigma^2_{e2} + k\,\sigma^2_{P(FS)xY} + k\,\sigma^2_{F(S)xY} + k\,\Theta^2_Y$
S x Y	3	$\sigma^2_{e2} + k\,\sigma^2_{P(FS)xY} + k\,\sigma^2_{F(S)xY} + k\,\Theta^2_{SxY}$
F(S) x Y	75	$\sigma^2_{e2} + k_2\,\sigma^2_{P(FS)xY} + k_3\,\sigma^2_{F(S)xY}$
P(FS) x Y	238	$\sigma^2_{e2} + k_1\,\sigma^2_{P(FS)xY}$
Residual	929	σ^2_{e2}
	Coefficients	
k_1	4.4379	
k_2	4.5600	
k_3	17.9789	
k_4	1.7077	
k_5	1.7172	
k_6	8.0735	
k_7	1.7180	
k_8	8.6524	
k_9	34.7237	

where $\sigma^2_{e2} = \sigma^2_{e1} + \sigma^2_{T(PFS)xY}$

Table 5.3. Least squares means and variances of offspring from breeding programs at California, Florida, Georgia and Queensland in 1990 and 1991 for average numbers of lesions per inoculated leaf (LS12), average leafspot severity rating (LSPOT), average length (mm) of stem cankers (CANKA) and stem canker appearance rating (CDA).

Trait	State	1990			1991		
		Mean		Variance	Mean		Variance
LS12	CA	5.95	b[z]	358.67	21.37	ab	810.63
(lesions/leaf)	FL	13.14	a	455.71	23.46	a	1185.13
	GA	4.72	b	297.75	12.86	c	692.64
	QL	15.57	a	557.02	19.15	b	1165.55
LSPOT	CA	0.68	b	4.61	3.62	a	8.56
(0-5)	FL	1.17	a	6.12	3.65	a	13.69
	GA	0.26	c	3.44	2.35	b	7.13
	QL	1.37	a	6.91	3.72	a	14.12
CANKA	CA	16.31	a	217.12	25.28	a	416.79
(mm)	FL	11.35	b	296.48	14.01	c	661.11
	GA	9.49	b	148.47	13.49	c	334.09
	QL	15.30	a	307.82	18.80	b	622.61
CDA	CA	4.77	a	5.63	4.60	a	5.33
(1-5)	FL	4.67	a	7.79	4.10	b	8.36
	GA	3.74	b	3.81	3.64	c	4.34
	QL	4.65	a	(8.31)	4.32	ab	8.06

[z]Mean separation by multiple t-test at $P \leq 0.05$.

Table 5.4. Half-sib family analysis of variance for 1990 and 1991 for average number of lesions per inoculated leaf (LS12), leafspot severity rating (LSPOT), average length (mm) of inoculated stem cankers (CANKA) and stem canker appearance rating (CDA).

		1990		1991	
Trait	Source	MS	F	MS	F
LS12	State	10332.499	30.427**	8056.875	10.754**
	Family (S)	339.585	1.094^{NS}	749.216	1.967**
	Plot (FS)	310.465	2.465**	380.799	1.244**
	Error	125.959		306.126	
LSPOT	State	88.220	22.170**	176.842	22.286**
	Family (S)	3.980	1.556**	7.935	2.206**
	Plot (FS)	2.558	2.180**	3.597	2.888**
	Error	1.173		1.245	
CANKA	State	3416.707	19.801**	8955.425	24.302**
	Family (S)	172.551	2.714**	368.512	3.775**
	Plot (FS)	63.568	1.382**	97.629	0.965^{NS}
	Error			101.180	
CDA	State	71.147	15.837**	57.003	11.907**
	Family (S)	4.493	2.549**	4.788	2.889**
	Plot (FS)	1.762	1.970**	1.657	1.245**
	Error	0.895		1.332	

^{NS},** Nonsignificant at $P \leq 0.05$ or significant at $P \leq 0.01$.

Table 5.5. Half-sib family analysis of variance of combined years for average number of lesions per inoculated leaf (LS12), leafspot severity rating (LSPOT), average length (mm) of inoculated stem cankers (CANKA) and stem canker appearance rating (CDA).

	LS12		LSPOT		CANKA		CDA	
Source	MS	F	MS	F	MS	F	MS	F
State	13633.587	18.411**	231.412	24.926**	11401.911	25.759**	124.382	17.663**
Family (S)	740.495	1.739**	9.284	2.668**	442.636	4.241**	7.042	3.002**
Plot (FS)	425.735	1.657**	3.480	2.040**	104.372	1.128^{NS}	2.346	1.591**
Tree (PFS)	256.957	1.590**	1.706	7.436**	92.544	1.957**	1.475	2.509**
Year	62835.185	180.102**	4289.495	1688.778**	16956.968	181.385**	48.349	23.156**
S x Y	4089.324	11.721**	18.272	7.194**	1191.648	12.747**	8.501	4.115**
F(S) x Y	348.887	1.361*	2.540	0.949^{NS}	93.486	1.698**	2.088	2.186**
P(FS) x Y	256.417	1.587**	2.678	5.722**	55.062	1.164*	0.955	1.624**
Residual	161.570		0.468		47.288		0.588	

^{NS},*,** Nonsignificant or significant at P $\leq$0.05 and 0.01, respectively.

Table 5.6. Estimates of variance components (and percent of total) from combined years half sib analysis for average number of spots per inoculated leaf (LS12), average leafspot severity rating (LSPOT), average length (mm) of inoculated stem cankers (CANKA) and stem canker appearance rating (CDA).

Variance component	LS12		LSPOT		CANKA		CDA	
F(S)	8.716	(3.2)	0.134	(6.2)	9.717	(11.0)	0.134	(8.9)
P(FS)	20.840	(7.6)	0.338	(15.6)	1.434	(1.6)	0.107	(7.2)
T(PFS)	55.857	(20.5)	0.725	(33.5)	26.501	(29.8)	0.519	(34.8)
F(S) x Y	4.998	(1.8)	-0.011	(0)	2.125	(2.4)	0.062	(4.2)
P(FS) x Y	21.372	(7.8)	0.498	(23.0)	1.752	(2.0)	0.083	(5.5)
Error	161.570	(59.1)	0.468	(21.7)	47.288	(53.2)	0.588	(39.4)

Table 5.7. Narrow sense heirtability estimates (± SE) from variance component analysis of half sib families for 1990, 1991 and combined years for average number of spots per inoculated leaf (LS12), leafspot severity rating (LSPOT), average length of inoculated stem cankers (CANKA) and stem canker appearance rating (CDA).

	Variance estimates of heritability[z]		
Trait	1990	1991	Combined
LS12	0.029 ± 0.045	0.234 ± 0.073	0.128 ± 0.037
LSPOT	0.197 ± 0.070	0.455 ± 0.101	0.248 ± 0.051
CANKA	0.435 ± 0.101	0.510 ± 0.107	0.438 ± 0.068
CDA	0.488 ± 0.108	0.435 ± 0.099	0.359 ± 0.062

[z]Individual tree narrow sense heritabilities (h^2) estimated as:

For single years $h^2 = \dfrac{4\sigma^2_{F(S)}}{\sigma^2_{F(S)} + \sigma^2_{P(FS)} + \sigma^2_E}$.

For combined years $h^2 = \dfrac{4\sigma^2_{F(S)}}{\sigma^2_{F(S)} + \sigma^2_{P(FS)} + \sigma^2_{T(FFS)} + \sigma^2_{F(S)xY} + \sigma^2_{P(FS)xY} + \sigma^2_E}$.

Table 5.8. Narrow sense heritability estimates (± SE) from regression of individual offspring values on the mean of the female parent for 1990, 1991 and combined years for average number of spots per inoculated leaf (LS12), leafspot severity rating (LSPOT), average length of inoculated stem cankers (CANKA) and stem canker appearance rating (CDA).

	Regression estimates of heritability[z]		
Trait	1990	1991	Combined
LS12	0.054 ± 0.057	0.187 ± 0.029	0.155 ± 0.022
LSPOT	0.716 ± 0.103	0.594 ± 0.093	0.496 ± 0.060
CANKA	1.156 ± 0.097	1.295 ± 0.089	1.054 ± 0.061
CDA	0.023 ± 0.088	0.876 ± 0.043	0.410 ± 0.059

[z]Narrow sense heritabilities obtained by multiplying the single parent-offspring regression coefficient by two.

Table 5.9. Narrow sense heritability estimates (± SE) from the total population of all four states for 1990 and 1991 (i.e. using σ^2_F instead of $\sigma^2_{F(S)}$).

	Total population heritabilities[z]		
Trait	1990	1991	Combined
LS12	0.469 ± 0.106	0.381 ± 0.092	0.301 ± 0.060
LSPOT	0.582 ± 0.119	0.946 ± 0.150	0.807 ± 0.114
CANKA	0.817 ± 0.143	0.948 ± 0.150	0.795 ± 0.113
CDA	0.838 ± 0.145	0.646 ± 0.123	0.645 ± 0.099

[z]Individual tree narrow sense heritabilities (h^2) calculated as:

For single year $h^2 = \dfrac{4\sigma^2_F}{\sigma^2_F + \sigma^2_{F(F)} + \sigma^2_{remainder}}$.

For combined years $h^2 = \dfrac{4\sigma^2_F}{\sigma^2_F + \sigma^2_{FxY} + \sigma^2_{remainder}}$.

Table 5.10. Phenotypic, genetic and environmental correlations (± SE) for 1990, 1991 and combined years between leafspot traits (LS12 and LSPOT) and stem canker traits (CANKA and CDA) estimated from half sib analysis of variance.

Traits		1990	1991	Combined
LS12/CANKA	r_P[z]	0.202 ± 0.024	0.111 ± 0.029	0.139 ± 0.023
	r_G	0.638 ± 0.488	0.930 ± 0.108	0.872 ± 0.094
	r_E	0.176	-0.344	-0.106
LS12/CDA	r_P	0.174 ± 0.014	0.049 ± 0.021	0.094 ± 0.015
	r_G	0.253 ± 0.408	0.660 ± 0.148	0.536 ± 0.141
	r_E	0.204	-0.245	-0.035
LSPOT/CANKA	r_P	0.093 ± 0.025	0.174 ± 0.033	0.144 ± 0.024
	r_G	0.572 ± 0.170	0.632 ± 0.112	0.619 ± 0.098
	r_E	-0.110	-0.253	-0.175
LSPOT/CDA	r_R	0.128 ± 0.017	0.191 ± 0.026	0.165 ± 0.018
	r_G	0.432 ± 0.177	0.658 ± 0.112	0.574 ± 0.106
	r_E	-0.009	-0.183	-0.074

[z] r_P, r_G and r_E are phenotypic, genetic and environmental correlations, respectively.

Table 5.11. Response per generation for selection of bacterial spot resistance on an individual tree basis for number of lesions per inoculated leaf (LS12), leafspot severity (LSPOT), length of inoculated cankers (CANKA) and stem canker appearance rating (CDA).

	LS12 (lesions/ leaf)	LSPOT (0-5)	CANKA (mm)	CDA (1-5)
Pooled h^2[z]	0.086	0.346	0.695	0.494
σ_P	16.533	1.471	9.424	1.222
Response[y] i = 0.798	1.135	0.406	5.227	0.482
Response[x] i = 1.400	1.990	0.713	9.169	0.845

[z]Pooled h^2 (unitless) from combined years estimate (Table 5.7) and parent-offspring regression estimate obtained by regressing 1990 offspring means on 1991 parent means and vice versa.

[y]Response when 50% of population is selected. i = selection intensity.

[x]Response when 20% of population is selected. i = selection intensity.

CHAPTER 6

COMBINING ABILITIES OF FIVE JAPANESE PLUM CULTIVARS FOR RESISTANCE TO *XANTHOMONAS* STEM CANKER.

Introduction

Stem cankers on Japanese plum (*Prunus salicina* Lindl. and hybrids) caused by *Xanthomonas campestris* pv. *pruni* (Smith) Dye lead to limb death or breakage and also provide a source of overwintering inoculum for spring infection (Feliciano and Daines, 1970; Moffett, 1973). Previous studies involving variance component estimation have shown that resistance to stem cankers is moderately to highly heritable and genetically correlated with leaf spot resistance (see Chapters 4 and 5). Accurate estimation of variance components and associated genetic parameters requires large populations and involves many assumptions about the selection of parents and the population from which they were derived (Cockerham, 1963). Combining abilities are based on first order statistics (means and sums) and so provide more robust estimates of inheritance than studies involving variance estimation (Gilbert, 1973; Simmonds, 1979). Diallel mating designs allow production of full-sib families for estimation of parental combining abilities and have been used to provide information on inheritance of disease

resistance in fruit trees (Quamme et al., 1990). If a fixed effects model is used, no genetic assumptions about the population or mating system are required. Combining abilities therefore offer several advantages in genetic studies. Four biparental families planted among the half-sib families in the Chapter 5 experiment segregated in 1990 in 1:1 and 3:1 ratios of susceptible:resistant suggesting a single gene controlling stem canker resistance. The purpose of this study was to simultaneously analyze a set of diallel crosses in both a quantitative manner by estimating combining abilities, and in a qualitative manner by testing the hypothesis of a single recessive gene for stem canker resistance.

Materials and Methods

The Japanese plum cultivars 'Burbank', 'Friar', 'Gulfruby', 'Wade' and 'Wilson' were hybridized at Applethorpe, Queensland, Australia to produce 9 of the 10 full-sib families for a complete half diallel. 'Wilson' is pollen sterile and so was used only as a seed parent. 'Gulfruby' flowers approximately 5 weeks before the other cultivars at Applethorpe and so was used exclusively as a pollen parent. Seed were stratified, germinated and planted in 5 liter pots in a greenhouse in Gainesville, Florida in 1990 and were tested for resistance to *Xanthomonas* stem canker in June 1991 when 12 months old. The 249 trees were arranged in a randomized complete block design. Actively growing shoots were inoculated at three sites within the top 15 cm with 2.5×10^8 cfu per ml of *X. campestris* pv. *pruni*

using a 26 gauge needle and syringe. Canker length was measured at 14 days and 28 days (CANKA) after inoculation. The difference in canker length at 14 and 28 days was analyzed as the canker expansion rate (DIFF) in mm per day. The appearance of the cankers was rated at 28 days (CDA) using a 1 (clean) to 5 (dirty) scale. Classes 1, 2, and 3 were considered resistant, and 4 and 5 were susceptible.

Estimates of combining ability were obtained by two methods as follows:

Method A Values for the missing cross ('Wilson' x 'Friar') were imputed from arithmetic averages of the other crosses and the five-parent diallel analyzed by Griffing's Method 4 Model 1 (Griffing, 1956) using a SAS Macro written by S.B. Linda (IFAS Statistics Department, University of Florida, Gainesville. Florida, 32611). Harmonic means were used for the average number of trees because of the unbalanced design (Hallauer and Miranda Fo, 1981).

Method B The incomplete diallel was analyzed as a fixed effects model corresponding to Method 4 Model 1 of Griffing (1956) using ordinary least squares with a sum to zero reparameterization to obtain unbiased estimates of general and specific combining ability.

Segregation for single gene hypothesis was tested using the chi square test with a correction for continuity applicable for single degree of freedom tests (Steel and Torrie, 1980).

Results and Discussion

Crosses differed in mean canker length, canker appearance rating and rate of canker extension (Table 6.1). There were no block effects indicating that the greenhouse environment was uniform in its influence on canker development. 'Friar' x 'Gulfruby' was the most susceptible cross and 'Burbank' x 'Wade' the most resistant as measured by all three traits (Table 6.2). Three variables were used to measure resistance because of the difficulty in describing the many forms of resistance that can occur with a single variable and the change in family ranking that may occur depending how resistance is measured. Length of inoculated cankers after 6 weeks has been shown to be moderate to highly repeatable in the field (Topp et al., 1991) and the canker expansion rate is a linear function of two such estimates and so should be of similar repeatability.

The favorable environment that existed in the greenhouse for canker development coupled with the high concentration of initial inoculum may have resulted in watersoaking or canker development in even the most resistant trees. Canker expansion rate between 14 and 28 days was used to avoid this possible

complication. The rationale was that even though the most resistant trees in this population might initially develop canker symptoms, a decrease in the rate of canker development would be a component of resistance (Parlevliet, 1979). The ranking of families for CANKA and DIFF were similar, but greater separation of classes was possible using DIFF as indicated by more significant differences among crosses (Table 6.4). The coefficient of variation for CDA (24.6%) was smaller than for CANKA (49.0%) and DIFF (74.3%) indicating that CDA had comparatively less variation among seedlings within each cross-block combination.

'Wilson' x 'Wade' had a higher value for CANKA than would have been predicted by the phenotypes of the parents (Table 6.2). This may be a result of the small number (14) of seedlings representing the family. One aberrant seedling in a small family can seriously bias the family mean. Larger families are beneficial because they reduce sampling error and avoid the complications of stray seedlings that may exist in a family due to errors during seed handling or pollination.

Differences among the diallel crosses were partitioned into components due to general combining ability (GCA) and specific combining ability (SCA) (Table 6.3). GCA measures the average performance of a parent in hybrid combination with all other parents in the diallel (Sprague and Tatum, 1942). The highly significant GCA term in Table 6.3 indicates that statistically additive features of the parents were important in explaining the differences among crosses. The two

methods used to calculate GCA estimates gave similar results (Table 6.4) for all traits. Method B using least squares provides unbiased estimates because no data imputation was necessary. Less similarity of results would have been expected from a population where SCA effects were of greater significance. Negative GCA values in Table 6.4 are desirable because they indicate smaller canker lengths, lower CDA ratings or slower canker expansion rate. 'Friar' and 'Gulfruby' were the most potent parents for transmitting susceptibility to cankers. 'Burbank', 'Wade' and 'Wilson' produced offspring with significantly smaller canker lengths than 'Friar,' but were not significantly different from each other in the canker length transmitted to their offspring (Table 6.4).

GCA parental ranking changed depending on which trait was used to measure resistance. Resistance as measured by canker appearance indicated that 'Wade' was superior to the other parents, transmitting on average a rating of 0.666 (on 1 to 5 scale) below the mean. Resistance as measured by canker length classed 'Burbank', 'Wade' and 'Wilson' in the same category (Table 6.4). Although trees with clean (CDA = 1 to 3) canker appearance ratings generally also had small cankers, there were exceptions. These exceptions appeared to occur more frequently in the greenhouse than in similar experiments conducted in the field. This may have been due to the rapid stem growth in the greenhouse at the time of inoculation which sometimes resulted in long stem scars (rather than

cankers) even for low CDA ratings. For this reason CDA rating was considered a more useful measure of resistance in the greenhouse. A selection index that combined both variables would be the best selection criterion.

All data were analyzed using the fixed effects model 1 of Griffing (1956) because parents were deliberately chosen from the Queensland Department of Primary Industries' (QDPI) Japanese plum breeding program and were selected to represent a range of susceptibilities to stem cankers. The main objective was estimation of GCA and SCA effects. These estimates are specific for the combination of parents tested. For example using a very susceptible set of parents of similar susceptibility to 'Friar' to estimate the GCA of 'Gulfruby' would result in a higher GCA estimate for 'Gulfruby' because the estimates are expressed as deviations from the population mean. Breeders face the problem of wishing to use GCA estimates across a broad population but not having the resources to test large numbers of parents. A compromise is to select genetically broad based material which is likely to represent future populations. Although only five parents were used in this diallel, they encompassed a range of susceptibilities in the QDPI program which should improve the utility of the GCA estimates.

SCA measures the deviation of a particular cross from the expected value based on GCA estimates alone (Sprague and Tatum, 1942). SCA was not signi-

ficant indicating that statistically non-additive effects were not important in explaining the differences among cross mean values (Table 6.5). Quamme et al. (1990) reported that SCA effects for bacterial fireblight resistance in pear as measured by length of stem blight were also nonsignificant.

Crosses were also analyzed for segregation of a single recessive gene for resistance (Table 6.6). CDA classes 1 to 3 were classified as resistant reactions and classes 4 and 5 as susceptible. Six of the nine crosses conformed to a single gene hypothesis. For the crosses 'Burbank' x 'Gulfruby', 'Burbank' x 'Friar' and 'Friar' x 'Gulfruby' there was a paucity of resistant offspring. 'Burbank' x 'Friar' (22:1) and 'Friar' x 'Gulfruby' (59:4) more closely resembled a 15:1 segregation expected from a digenic model. Alternatively, 'Friar' may be homozygous susceptible and the segregants in these two crosses (5 out of 86) mislabelled seedlings. Frequency distributions for crosses involving the resistant parent 'Wade' indicate there may be single gene segregation with distributions of resistant offspring about the CDA = 2 rating and susceptible offspring about the CDA = 4 and 5 ratings (Figure 6.1).

The number of individuals per cross is small for most families, and so it is difficult to make conclusions on these data regarding the existence of a single resistance gene. Another possibly complicating factor is the method of seed production. Tents were erected over seed trees to prevent contamination by

insects, but flowers were not emasculated. Japanese plums are mostly self-infertile but some genotypes will self-pollinate (Weinberger, 1975). The single gene hypothesis needs to be tested using seed produced under stringent controls specifically for genetic testing and should include F_1 and backcross families of sufficient size to allow valid tests.

Summary and Conclusions

Analysis of a five parent diallel in a greenhouse provided general and specific combining ability estimates for resistance to *Xanthomonas* stem canker as measured by length of inoculated cankers, canker appearance rating and canker expansion rate. 'Friar' and 'Gulfruby' were the most susceptible parents. 'Burbank', 'Wilson' and 'Wade' had similar GCA values for length of inoculated cankers, but 'Wade' was the superior parent in transmitting canker resistance as measured by canker appearance rating. SCA was not important in determining the performance of a cross. Canker appearance rating was a better measure of resistance in the greenhouse tests due to a lower coefficient of variation and greater separation of GCA estimates. Segregation patterns for CDA were inconclusive in determining single gene control of resistance and require further study. 'Wade' segregated as a homozygous recessive resistant genotype in all crosses. 'Friar' segregated as though heterozygous at one critical locus in one cross ('Wade' x 'Friar') but more closely followed a digenic model in two crosses.

Table 6.1. Observed mean squares from randomized complete block analyses of variance for length (mm) of inoculated cankers (CANKA), canker appearance rating on 1-5 scale (CDA) and rate (mm/day) of canker expansion (DIFF). Mean and CV are presented for CANKA, CDA, DIFF.

		Mean squares		
Source	df	CANKA	CDA	DIFF
Cross	8	454.334**	7.567**	1.199**
Block	2	122.265^{NS}	1.577^{NS}	0.225^{NS}
Cross x Block	16	73.056^{NS}	1.749^{NS}	0.228^{NS}
Error	222	58.064	1.125	0.157
Mean		15.558	4.305	0.533
CV (%)		49.0	24.6	74.3

^{NS},** F tests not significant at $P \leq 0.05$ or significant at $P \leq 0.01$, respectively.

Table 6.2. Cross means for length (mm) of inoculated cankers (CANKA), canker appearance rating on 1-5 scale (CDA) and rate (mm/day) of canker expansion (DIFF).

Cross	Trees (no.)	CANKA[z] (mm)		CDA[z] (1-5)		DIFF[z] (mm/day)	
Wilson x Burbank	13	12.4	b	3.9	abc	0.36	c
Wilson x Wade	14	14.5	b	3.7	bc	0.43	bc
Wilson x Gulfruby	5	13.7	b	4.0	abc	0.39	c
Burbank x Friar	23	16.5	ab	4.7	a	0.56	abc
Burbank x Wade	21	11.1	b	3.3	c	0.33	c
Burbank x Gulfruby	79	12.5	b	4.5	ab	0.38	c
Friar x Wade	9	16.7	ab	3.7	bc	0.74	ab
Friar x Gulfruby	63	21.8	a	4.8	a	0.85	a
Wade x Gulfruby	22	14.4	b	3.8	bc	0.46	bc

[z]Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

Table 6.3. Mean squares from combining ability analyses, based on method 4 model 1 of Griffing (1956) for length (mm) of inoculated cankers (CANKA), canker appearance rating on 1-5 scale (CDA) and rate (mm/day) of canker expansion (DIFF).

Source	df[z]	Mean squares		
		CANKA	CDA	DIFF
General combining ability	4	16.813**	0.431**	0.0597**
Specific combining ability	5	2.940[NS]	0.076[NS]	0.0063[NS]
Error	222	4.147	0.080	0.0112

[NS],** Not significant at P $\leq$0.05 and significant at P $\leq$0.01, respectively.

[z]Analysis using imputed data for cross of Wilson x Friar.

Table 6.4. Estimates of general combining ability effects obtained by analysis of variance (method A) and ordinary least squares (method B) for length (mm) of inoculated cankers (CANKA), canker appearance rating on 1-5 scale (CDA) and rate (mm/day) of canker expansion (DIFF).

	Method A[z]			Method B[z]		
Parent	CANKA (mm)	CDA (1-5)	DIFF (mm/day)	CANKA (mm)	CDA (1-5)	DIFF (mm/day)
Burbank	-2.425 c	0.068 ab	-0.1310 b	-2.867 c	0.017 ab	-0.1483 b
Friar	3.683 a	0.369 a	0.2290 a	4.227 a	0.431 a	0.2577 a
Gulfruby	0.857 ab	0.284 ab	0.0180 b	0.400 b	0.268 ab	-0.0089 b
Wade	-1.047 bc	-0.576 c	-0.0196 b	-1.483 bc	-0.666 c	-0.0326 b
Wilson	-1.068 bc	-0.146 bc	0.0964 b	-0.277 bc	-0.050 b	-0.0679 b

[z]Means separation within columns by lsd at P ≤0.05.

Table 6.5. Estimates of specific combining ability for each cross obtained by analysis of variance (method A) and ordinary least squares (method B) for length (mm) of inoculated cankers (CANKA), canker appearance rating on 1-5 scale (CDA) and rate (mm/day) of canker expansion (DIFF).

	Method A			Method B		
Cross	CANKA (mm)	CDA (1-5)	DIFF (mm/day)	CANKA (mm)	CDA (1-5)	DIFF (mm/day)
Wilson x Burbank	0.969	-0.052	0.0805	0.432	-0.156	0.0591
Wilson x Wade	1.680	0.383	0.0423	1.098	0.294	0.0212
Wilson x Gulfruby	-1.015	-0.191	-0.0398	-1.530	-0.138	-0.0803
Burbank x Friar	0.275	0.249	-0.0430	-0.227	0.202	-0.0697
Burbank x Wade	-0.361	-0.260	-0.0268	0.126	-0.154	-0.0108
Burbank x Gulfruby	-0.883	0.063	-0.0300	-0.331	0.107	0.0214
Friar x Wade	-0.929	-0.180	0.0107	-1.429	-0.187	0.0001
Friar x Gulfruby	2.289	0.071	0.0960	1.656	-0.016	0.0695
Wade x Gulfruby	-0.390	0.057	-0.0454	0.205	0.047	-0.0106
lsd (.05) 1 common parent	4.609	0.642	0.2395	4.609	0.642	0.2395
lsd (.05) no common parents	3.259	0.454	0.1694	3.259	0.454	0.1694

Table 6.6. Segregation ratios for canker appearance rating (CDA) on 1-5 scale.

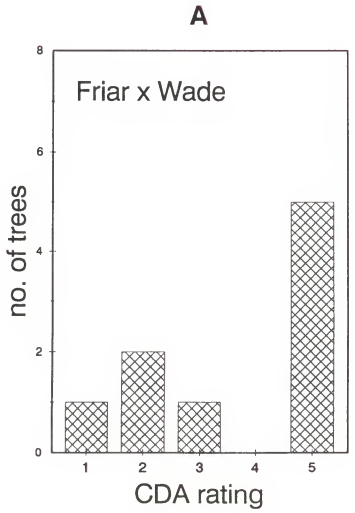
Cross	Observed[z]	Expected[y]	χ^2	Probability
Wilson x Gulfruby	3:2	3:1	0.067	.75 - .90
Wilson x Wade	9:5	1:1	0.643	.25 - .50
Wilson x Burbank	8:5	3:1	0.641	.25 - .50
Burbank x Gulfruby	69:10	3:1	5.776	.01 - .05
Burbank x Wade	9:12	1:1	0.191	.50 - .75
Burbank x Friar	22:1	3:1	4.188	.01 - .05
Friar x Gulfruby	59:4	3:1	10.714	<.01
Friar x Wade	5:4	1:1	0.111	.50 - .75
Wade x Gulfruby	13:9	1:1	0.409	.50 - .75

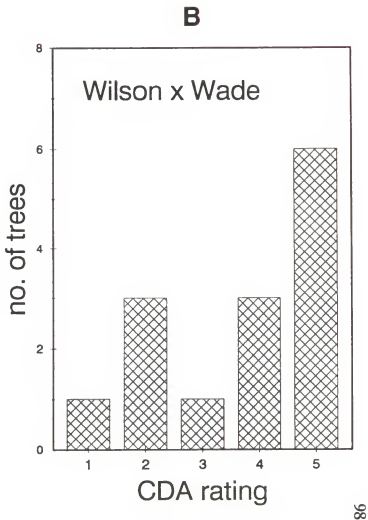
[z]Observed ratio with susceptible rating of 4 or 5 on CDA scale: resistant rating of 1-3 on CDA scale.

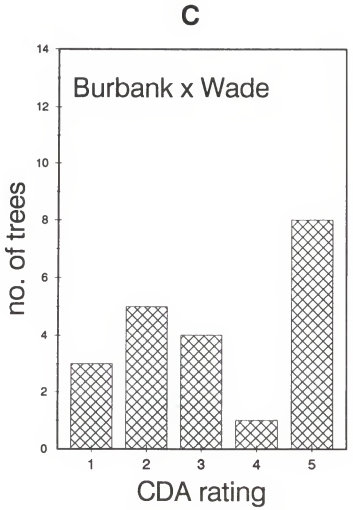
[y]Expected ratio with one recessive gene for resistance and predicted genotypes of Wade (homozygous recessive); Burbank, Gulfruby, Friar and Wilson (heterozygous).

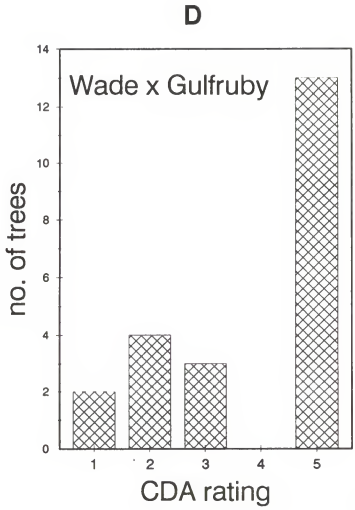
Figure 6.1. Frequency distributions for canker appearance rating (CDA) for crosses using the resistant parent Wade. CDA values 1 to 3 are resistant, and CDA values 4 to 5 are susceptible.

A) Friar x Wade
B) Wilson x Wade
C) Burbank x Wade
D) Wade x Gulfruby


A
Friar x Wade
no. of trees
0
2
4
6
8
1
2
3
4
5
CDA rating


B
Wilson x Wade
no. of trees
0
2
4
6
8
1
2
3
4
5
CDA rating


C
Burbank x Wade
no. of trees
0
2
4
6
8
10
12
14
1
2
3
4
5
CDA rating


D
Wade x Gulfruby
no. of trees
0
2
4
6
8
10
12
14
1
2
3
4
5
CDA rating

CHAPTER 7

GROWTH OF *XANTHOMONAS CAMPESTRIS* PV. *PRUNI* IN RESISTANT VERSUS SUSCEPTIBLE JAPANESE PLUM CLONES

Introduction

Japanese plum (*Prunus salicina* Lindl. and hybrids) clones with varying levels of resistance to leaf spot and stem canker caused by *Xanthomonas campestris* pv. *pruni* (Smith) Dye were identified in Chapter 4. Disease pressure in the field was low and several clones appeared resistant to leafspot in 1990, but in 1991 disease pressure was very high and 'Segundo' and 'Robusto' were noted to have outstanding leaf resistance. Quantification of the pathogen's effect on the host by measurement of plant response such as leaf, stem, or fruit symptoms is the common method of assessing disease resistance. However, there are many environmental factors which may be confounded with measurement of disease resistance in this manner. Topp et al. (1991) reported clonal repeatabilities of about 0.5 for measurement of bacterial spot symptoms indicating there was significant variation among ramets of the one clone due to confounding environmental factors. Plant resistance to disease can also be assessed by direct measurement of the growth and development of the pathogen. Reduced or delayed pathogen

development in the host plant is equated with resistance (Parlevliet, 1989). Hammerschlag (1988a) used *X. campestris* pv. *pruni* concentration in stem pieces for in vitro screening of peach bacterial spot resistance. Graham et al. (1990) used bacterial concentration in citrus leaves to characterize the interactions of citrus cultivars with strains of *X. campestris* pv. *citrumelo*. The purpose of this study was to measure bacterial populations in stems and leaves of plum clones that had been previously classified as resistant and susceptible by measurement of disease symptoms in the field.

Materials and Methods

Inoculum Preparation

A rifamycin resistant strain of *X. campestris* pv. *pruni* was grown overnight in Difco nutrient broth, pelleted by centrifugation and resuspended in sterile tap water to obtain 5 x 10^8 cfu per ml by photometrically standardizing to 0.3 A at 600 μm wavelength. These suspensions were serially diluted with sterile tap water to obtain the specified concentrations.

Stem Bacterial Populations

'Royalbeaut', 101, 'Rubysweet' and 'Wade' plum clones were inoculated in the field in May 1991 with 5 x 10^6 cfu per ml of the bacteria. Four branches per clone were inoculated using a 26 gauge needle and syringe at three sites per

branch within the top 15 cm of actively growing shoots. One branch per clone was harvested at 7, 15, 21 and 31 days after inoculation. Branches were cut into 10 mm pieces centered around the inoculation sites. Each stem piece was crushed in 1 ml of sterile tap water, the suspension was serially diluted in sterile tap water, and 0.05 ml subsamples of the final dilution were spread on rifamycin (50 ppm) containing nutrient agar plates. Bacterial colonies were counted after incubation at 28°C for 3 days. Colony counts were converted to $\log_{10}$(cfu/10 mm stem piece + 1) for all analyses.

Leaf Bacterial Populations

Fully expanded leaves between the fourth and seventh leaf (counting from the shoot tip) of the plum clones 'Blackamber', C333-1, 'Robusto' and 'Segundo' were inoculated in August 1991 in the field with 5×10^5 cfu per ml of the bacteria. C333-1 is the code for a seedling from selection BY8351-16 from the United States Department of Agriculture (USDA) plum breeding program at Byron, Georgia. Leaves were infiltrated using a needleless syringe to produce a visibly watersoaked circle of approximately 10 mm diameter. Thirty sites per clone were inoculated comprising three sites per leaf, two leaves per branch and five branches per clone. One branch per clone was harvested at 0, 3, 7, 14 and 20 days after inoculation. Leaf disks (0.32 cm^2) from within the watersoaked area were removed with a corkborer and two disks (one from each leaf) were crushed in 1

ml of sterile tap water. The suspension was serially diluted in sterile tap water and 0.05 ml subsamples of the final dilution were spread on rifamycin (50 ppm) containing nutrient agar plates. Bacterial colonies were counted and analyzed as described for the stem bacterial populations.

Intercellular Volume

Leaf mesophyll susceptible clones 'Blackamber' and C333-1 were compared with resistant clones 'Robusto' and 'Segundo'. The third, fourth, seventh, tenth, and twelfth leaves from the shoot tip were collected for each plum clone. Three sites on one side of the midvein were infiltrated with tapwater using a needleless syringe. Leaf disks (0.32 cm^2) were cut from within the watersoaked area using a corkborer and immediately weighed. The procedure was repeated, but without infiltration, for the control side of the leaf and the difference in measurements used as a measure of intercellular volume. Mean intercellular volume for each clone consisted of 15 observations.

Field Resistance

Plum clones were evaluated in the field for stem and leaf resistance to *X. campestris* pv. *pruni* as described in Chapter 4. Leaf resistance was measured by the average number of lesions from leaves that had been dipped in 2.5 x 10^8 cfu per ml (LS12) and by a 0 (none) to 5 (heavy) rating of severity of leaf infection

following natural infection (LSPOT). Stem resistance was measured by the average length of three inoculated cankers (CANKA) and by a 1 (clean) to 5 (dirty) rating of the canker appearance (CDA). Means are presented for two years data and an average of 4 ramets per clone per year.

Results and Discussion

Stem Bacterial Populations

'Royalbeaut' and 101 were significantly more susceptible to stem cankers than 'Rubysweet' and 'Wade' as measured in the field by length of inoculated cankers (CANKA) and 'Royalbeaut' was significantly more susceptible than 'Rubysweet' as measured by canker appearance rating (CDA) (Table 7.1). 'Royalbeaut' is a commercial plum grown in California and 101 is a clone from the USDA breeding program at Fresno, California. Resistance to *X. campestris* pv. *pruni* is unnecessary in California due to the dry climate and very low incidence of disease. 'Rubysweet' and 'Wade' were bred in the southeastern United States where bacterial spot is a major problem and were released as resistant cultivars (Brooks and Olmo, 1983; Tehrani et al., 1991). The bacterial population 7 days after inoculation was similar for all clones, but there was clear separation of resistant and susceptible groups by day 21 ($P \leq 0.05$)(Figure 7.1). The susceptible clones reached a maximum concentration of approximately 10^7 cfu per 10 mm stem piece whereas the resistant clones did not support bacterial populations

above approximately 10^4 cfu per 10 mm stem piece. No bacteria were detected from day 21 to day 31 in 11 of 12 inoculation sites sampled for 'Wade' and 'Rubysweet'. The reduced bacterial population in resistant versus susceptible genotypes concurs with reports in citrus (Graham et al., 1990). It would be possible to screen for resistance to stem cankers by sampling inoculated sites after 30 days and culling genotypes with bacterial populations above 10^4 cfu per 10 mm stem piece. However, similar results at a much reduced cost could be obtained by simply measuring the length of cankers resulting from inoculation as was done with the field resistance screening.

A point which arose from this study and which has been confirmed by observation of canker lengths in the field is that there is variability within this system. The lower than expected value for 'Royalbeaut' at day 7 in Figure 7.1 resulted from two of the three replicates having no detectable levels of bacteria. Only a small volume of bacteria can be injected into the stem and escapes are possible. To minimize this problem we injected with a very high concentration (2.5×10^8 cfu per ml) in the field study in Chapter 4 and replicated the treatment on each genotype. In future bacterial population studies it would be advantageous to bulk two or three stem pieces for each replicate. This would reduce the variability among replicates within a genotype, but without increasing the number of serial dilutions and platings which are the most time consuming part of the method.

Leaf Bacterial Populations

'Blackamber' was significantly more susceptible in the field to *Xanthomonas* leaf spot than C333-1, 'Robusto' and 'Segundo' as measured by number of lesions per leaf following dipping of the leaf into a high concentration of the bacteria (LS12) and by the severity of leafspot infection after natural infection (LSPOT) (Table 7.2). Bacterial populations increased initially in all clones, but the rate of increase was slower for 'Robusto' and 'Segundo' so that after 7 days they had reached a concentration of approximately 10^5 cfu per cm^2 of leaf compared with approximately 10^8 for 'Blackamber' and C333-1 (Figure 7.2). 'Robusto' and 'Segundo' reached peak concentrations of bacteria at about day 14 after which the populations declined. Bacterial populations in 'Blackamber' and C333-1 reached peak concentrations at about the same time as in 'Robusto' and 'Segundo' but did not decline from day 14 to day 20. The initial increase in bacterial populations in the resistant as well as the susceptible clones suggests that the resistance response is induced (Klement, 1982).

High bacterial populations developed in leaves of C333-1 (10^8 cfu/ cm^2 of leaf) and yet C333-1 had low severity of leafspot infection in the field (LSPOT rating of 2). Possible explanations for this difference are:

1. There may be some epidermal level of resistance operating which is bypassed when the bacteria are injected directly into the mesophyll. A similar response was noted for the cultivar 'Bruce' in Chapter 3.

2. C333-1 may be exhibiting a tolerance to infection whereby disease symptoms are reduced despite high bacterial levels (Robinson, 1976).
3. The LSPOT field rating may differentiate between levels of quantitative resistance which are not associated with large differences in bacterial populations.

Parlevliet (1989) defines complete resistance as total prevention of pathogen growth. This is the type of resistance occurring in single gene hypersensitive responses (Flor, 1955). Different levels of quantitative resistance may occur which involve only slight reductions in pathogen numbers. It is possible that the LSPOT field rating is discriminating among genotypes on this basis. This would be similar to the base level of horizontal resistance described by Vanderplank (1963) as present in all plants.

C333-1 and 'Blackamber' were tested in Chapter 3 for leaf resistance by measurement of the percentage watersoaking 14 days after infiltration with 5×10^5 cfu per ml (INF5) and with 5×10^6 cfu per ml (INF6). The INF6 method ranked C333-1 and 'Blackamber' as equally susceptible which concurs with the population results in Figure 7.2. However, the INF5 method ranked C333-1 as less susceptible than 'Blackamber' but this may be explained by insufficient time for the full potential development of bacteria after only 2 weeks.

P. angustifolia Marsh. is the native 'hog' plum of the southeastern U.S.A. and has been used extensively as a source of resistance to *X. campestris* pv. *pruni* in breeding programs in this region (Topp and Sherman, 1990). Topp and Sherman (1990) reported *P. angustifolia* accounted for an average of 6% of the species composition of 18 resistant cultivars surveyed but was not in the eight susceptible cultivars. 'Segundo' and 'Robusto' contain five species in their ancestry but still have 25% *P. angustifolia* (Brooks and Olmo, 1982; Tehrani et al., 1991). It is possible that their high leaf resistance is derived from *P. angustifolia*.

Intercellular Volume

All clones were inoculated with the same concentration of bacteria, at the one time and using the same method and yet populations on the day of inoculation differed (Figure 7.2). 'Blackamber' and C333-1 had about 150 cfu per cm^2 of leaf whereas 'Robusto' and 'Segundo' contained about 20 cfu per cm^2 of leaf. The host resistance response may have drastically reduced the bacterial populations within the 3 hours between inoculation and sampling but this seems unlikely. 'Blackamber' and C333-1 had significantly greater intercellular volumes than 'Robusto' and 'Segundo' (Figure 7.3), which may account for the differences in initial bacterial populations. Leaf morphology associated with resistance to bacteria is not common but has been reported (Huang, 1986). Further experiments are planned to study the relationship between intercellular volume

and leaf resistance. It is also planned to study the inheritance of both leaf resistance and intercellular volume in segregating progenies of 'Robusto' and 'Segundo'. If the resistance of 'Robusto' and 'Segundo' is characterized by low intercellular volume, then it may be possible to initially screen segregating populations for resistance without the expense of inoculation with the pathogen.

Summary and Conclusions

High levels of *X. campestris* pv. *pruni* developed in stems (10^7 cfu per 10 mm stem piece) and leaves (10^8 cfu per cm^2 of leaf) of plum clones which had been classified as susceptible from field ratings. 'Rubysweet' and 'Wade' which were classed as resistant to stem cankers in the field had low levels of *X. campestris* pv. *pruni* (less than 10^5 cfu per 10 mm stem piece) 21 days after inoculation. C333-1 was classed as resistant to leaf spot in the field but supported high levels of bacteria in the leaf (about 10^8 cfu per cm^2 of leaf) when infiltrated with *X. campestris* pv. *pruni*. Several possible reasons are suggested for this difference. In contrast, bacteria did not reach high levels (less than 10^5 cfu per cm^2) in 'Robusto' and 'Segundo,' which were leaf resistant in the heavy infection year of 1991. The levels of resistance observed in these two clones offers great potential for breeding for resistance to leaf spot. It should be possible to screen for this type of resistance (i.e., resistance associated with low pathogen levels) by measuring bacterial populations 20 days after infiltration with 5×10^6 cfu per ml

inoculum or more simply by measurement of percentage watersoaking after 20 days. Stem canker resistance can be accurately assessed, without the need for bacterial population studies, by measurement of length of inoculated cankers, but adequate replication is essential. It is possible that the low to moderate repeatabilities obtained for various measures of leafspot resistance in Chapter 3 involved plum clones with only small differences in their bacterial population levels. In contrast, the leaf resistance in 'Robusto' and 'Segundo' is associated with large differences in pathogen populations and may be shown to be under simple genetic control.

Table 7.1. Mean field ratings over two years of plum clones for average length of inoculated cankers (CANKA), and canker appearance rating (CDA).

Clone	CANKA (mm)	CDA (1-5)
101	32.1 a[z]	3.9 ab
Royalbeaut	24.8 a	5.0 a
Wade	10.8 b	3.3 ab
Rubysweet	8.5 b	2.9 b

[z]Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

Table 7.2. Mean field ratings over two years of plum clones for average number of lesions per inoculated leaf (LS12), and severity of leafspot infection (LSPOT).

Clone	LS12 (lesions/leaf)	LSPOT (0-5)
Blackamber	56.4 a[z]	3.3 a
C333-1	8.2 b	2.0 b
Robusto	6.2 b	0.8 c
Segundo	5.9 b	1.3 bc

[z]Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

Figure 7.1. Bacterial populations in 10 mm stem pieces of plum clones Royalbeaut, 101, Rubysweet and Wade after inoculation with *X. campestris* pv. *pruni*. Each point represents the mean of three inoculations.

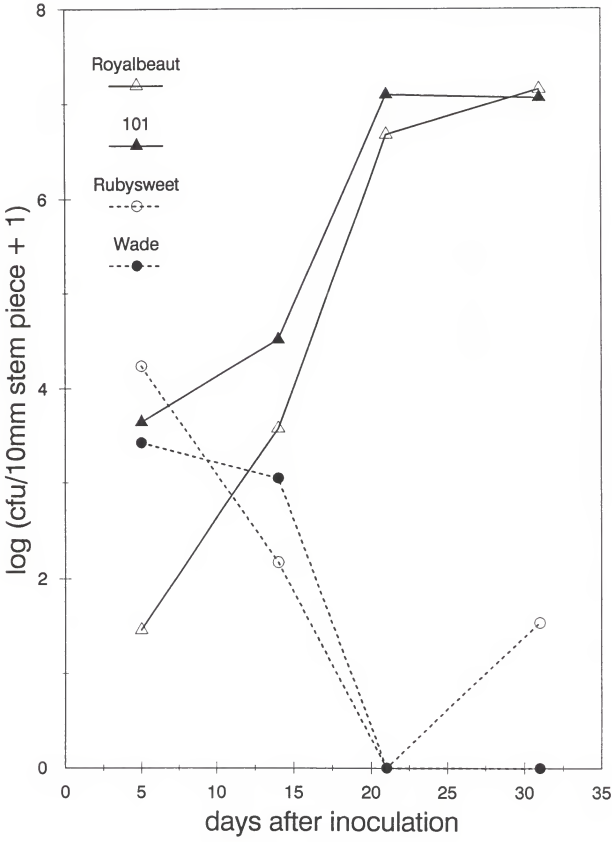

Royalbeaut
101
Rubysweet
Wade
log (cfu/10mm stem piece + 1)
days after inoculation
0
2
4
6
8
0
5
10
15
20
25
30
35

Figure 7.2. Bacterial populations in leaves of plum clones Blackamber, C333-1, Robusto and Segundo after inoculation with *X. campestris* pv. *pruni*. Each point represents the mean of six inoculation sites.

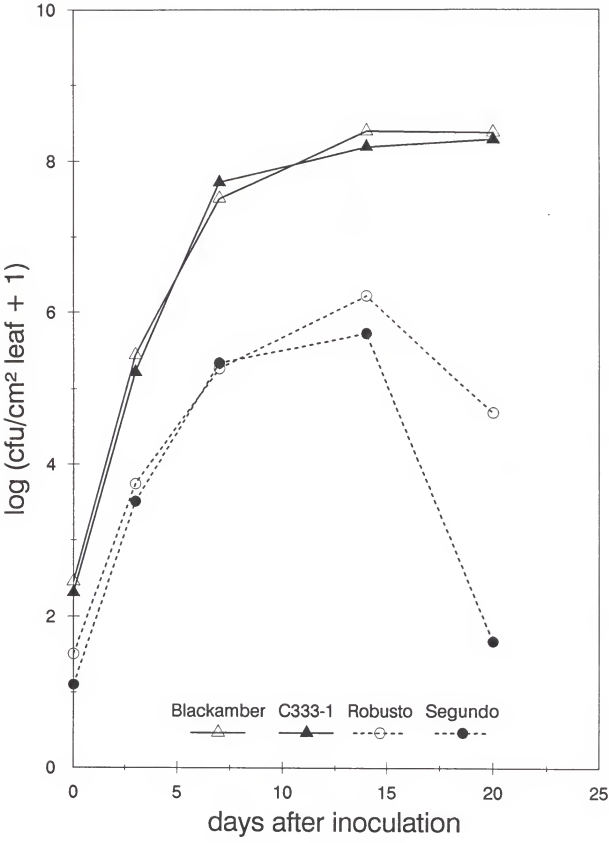
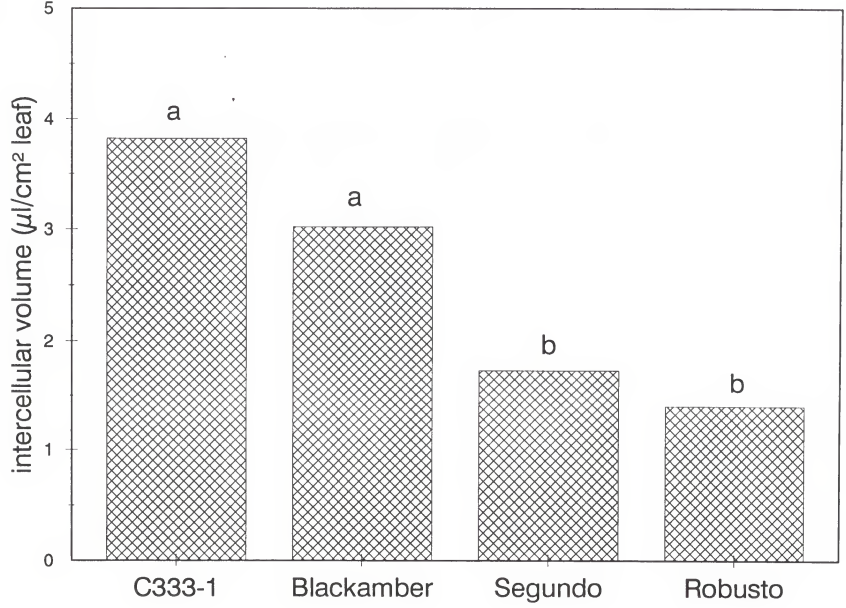

10
8
6
4
2
0
log (cfu/cm² leaf + 1)
0
5
10
15
20
25
days after inoculation
Blackamber
C333-1
Robusto
Segundo

Figure 7.3. Intercellular volume ($\mu l/cm^2$) of the plum clones C333-1, Blackamber, Segundo, and Robusto determined by difference in mass of infiltrated and control leaf disks. Bars represent the mean of 15 observations. Mean separation is by Duncan's multiple range test at $P \leq 0.05$.


intercellular volume (µl/cm² leaf)
0
1
2
3
4
5
a
a
b
b
C333-1
Blackamber
Segundo
Robusto

CHAPTER 8

SUMMARY AND CONCLUSIONS

The inheritance of resistance to *Xanthomonas campestris* pv. *pruni* in leaves and stems of Japanese-type plum (*Prunus salicina* and hybrids) was studied using 80 half-sib families and their clonal seed parents sampled from plum breeding programs at California, Georgia, Florida, and Queensland. The parent trees and half-sib seedlings were evaluated for two years in a disease nursery after artificial inoculation and natural spread of disease. Resistance in leaves was measured by the average number of lesions per leaf after dipping leaves in 2.5 x 10^8 cfu/ml inoculum (LS12) and by a 0 to 5 rating scale of leafspot severity following natural infection (LSPOT). Resistance in stems was measured by length (mm) of inoculated cankers (CANKA) and a 1 (clean) to 5 (dirty) rating of canker appearance (CDA). Breeding programs from the four states differed significantly in levels of bacterial spot on leaves and stems. Georgia germplasm was most resistant averaging 24.2 lesions/leaf for LS12 and 13.3 mm for CANKA, and California germplasm was most susceptible averaging 42.5 lesions/leaf for LS12 and 21.1 mm for CANKA.

Rainfall was much higher in early summer of 1991 than in 1990 and levels of bacterial spot increased dramatically in the wet year. The large difference in year effects provided for extreme tests of genotype x year interactions. Broad sense heritability (H_B) estimates for leaf resistance traits in 1991 were 0.5 to 0.6 and in 1990 the H_B for stem resistance traits were 0.5 to 0.6, but over combined years the H_B was only low to moderate (0.331 ± 0.053 for LS12; 0.206 ± 0.047 for LSPOT; 0.350 ± 0.054 for CANKA; and 0.371 ± 0.054 for CDA). The difference in H_B estimates between combined years and individual years was due to genotype x year interaction which was higher for leaf traits than for stem traits but significant for all traits. Estimates of H_B indicate that the traits measuring stem canker resistance are less influenced by environment than the LSPOT resistance trait.

Narrow sense heritabilities (h^2) were estimated by half-sib variance component analysis equating the variance among families within states to the genetic covariance among family members, by equating the variance among families (independent of state) to the genetic covariance, and by parent offspring regression. Estimates from the first method for combined years were 0.128 ± 0.030 for LS12, 0.248 ± 0.051 for LSPOT, 0.438 ± 0.068 for CANKA, and 0.359 ± 0.062 for CDA. These estimates indicate that greater gain will be possible when selecting for stem canker resistance than when selecting for leaf spot

resistance. Combined year h^2 estimates obtained from the other methods also ranked CANKA as most heritable and LS12 as least heritable. Estimates from parent offspring regression were higher than those from the first method, but may have been biased by nongenetic covariance arising from testing the parents and offspring in the same years and location. The genetic correlations (r_G) between leaf and canker resistance traits were high with $r_G = 0.872$ for LS12/CANKA and $r_G = 0.619$ for LSPOT/CANKA, indicating that there are common genes controlling both traits. Indirect selection for leaf spot resistance via selection for canker resistance would be an economical method of improving both traits.

General combining ability estimates indicated that 'Wade' is a good parent for stem canker resistance and that 'Gulfruby' and 'Friar' are poor parents. The hypothesis of a single recessive gene controlling stem canker resistance was supported from segregation data using 'Wade' as a parent but not from crosses involving 'Friar'.

High levels of *X. campestris* pv. *pruni* developed in stems (10^7 cfu per 10 mm stem piece) and leaves (10^8 cfu per cm^2 of leaf) of plum clones which had been classified as susceptible from field ratings. 'Rubysweet' and 'Wade' which were classed as resistant to stem cankers in the field had low levels of *X. campestris* pv. *pruni* (less than 10^5 cfu per 10 mm stem piece) 21 days after inoculation. Bacteria did not reach high levels (less than 10^5 cfu per cm^2) in

'Robusto' and 'Segundo' which were leaf resistant in the high infection year of 1991. The levels of resistance observed in these two clones offers great potential for breeding for resistance to leaf spot. It is possible that the low to moderate repeatabilities obtained for various measures of leafspot resistance in Chapter 3 involved plum clones with only small differences in their bacterial population levels. In contrast, the leaf resistance in 'Robusto' and 'Segundo' is associated with large differences in pathogen populations and may be shown to be under simple genetic control.

Resistance to leaf spot that is associated with reduced bacterial levels can be effectively screened by measuring bacterial populations 20 days after leaf infiltration with 10^6 cfu/ml inoculum, or more simply by measurement of percentage watersoaking. Resistance to stem cankers can be efficiently screened without the need for bacterial population studies. Inoculation of stems with 10^8 cfu/ml inoculum and measurement of canker length after 6 weeks is an efficient screen but adequate replication is essential to avoid selection of escapes.

LITERATURE CITED

Anderson, H.W. 1956. Diseases of fruit crops. McGraw-Hill, New York.

Becker, W.A. 1984. Manual of quantitative genetics. 4th ed. McNaughton and Gunn Inc., Ann Arbor, Mich.

Becker, W.A. 1985. Manual of quantitative genetics. 4th ed. Academic Enterprises, Pullman, Wash.

Bell, R.L., J. Janick, R.H. Zimmerman and T. van der Zwet. 1977. Estimation of heritability and combining ability for fire blight resistance in pear. J. Amer. Soc. Hort. Sci. 102:133-138.

Bohren, B.B., H.E. McKean and Y. Yamada. 1961. Relative efficiencies of heritability estimates based on regression of offspring on parent. Biometrics 17:481-491.

Brooks, R.M. and H.P. Olmo. 1982. Register of new fruit and nut varieties. List 32. HortScience 17:17-23.

Burton, G.W. and E.H. DeVane. 1953 Estimating heritability in tall fescue (*Festuca arundinacea*) from replicated clonal material. Agronomy Journal 45:478-481.

Campbell, C.L. and L.V. Madden. 1990. Introduction to plant disease epidemiology. John Wiley & Sons, New York.

Casler, M.D. 1982. Genotype x environment interaction bias to parent-offspring regression heritability estimates. Crop Sci. 22:540-542.

Chang, L.S., A. Iezzoni and G. Adams. 1991. Heritability of *Leucostoma peroonii* canker resitance among diverse peach genotypes. HortScience 26:60-62.

Civerolo, E.L. and H.L. Keil. 1976. Evaluation of *Prunus* spp. resistance to *Xanthomonas pruni* by artificial inoculation. Fruit Var. J. 30:17-18.

Clayton, C.N. 1976. Bacterial spot resistance in stone fruits. Fruit Var. J. 30:15-16.

Cockerham, C.C. 1963. Estimation of genetic variances, p. 53-94. In W.D. Hanson and H.F. Robinson (eds.). Statistical genetics and plant breeding. NAS-NRC 982. Washington, D.C.

Crow, J.F. 1986. Basic concepts in population, quantitative, and evolutionary genetics. W.H. Freeman and Co., New York.

Daines, R.H. and L.F. Hough. 1951. Artificial inoculation of peach seedlings with *Xanthomonas pruni*. Fruit Var. J. 41:8-9. (Abstr.)

Dunegan, J.C. 1932. The bacterial spot disease of the peach and other stone fruits. USDA Tech. Bull. No. 273, 51 p.

Du Plessis, H.J. 1984. Scanning electron microscopy of *Xanthomonas campestris* pv. *pruni* in plum petioles and buds. Phytopathol. Z., 109:277-284.

Du Plessis, H.J. 1986. Systemic migration and establishment of *Xanthomonas campestris* pv. *pruni* in plum leaves and twigs. J. Phytopathol. 116:221-227.

Du Plessis, H.J. 1987a. Canker development on plum shoots following systemic movement of *Xanthomonas campestris* pv. *pruni* from inoculated leaves. Plant Dis. 71:1078-1080.

Du Plessis, H.J. 1987b. Resistance of Laetitia plum to *Xanthomonas campestris* pv. *pruni*. Phytolactica 19:249-250.

Du Plessis, H.J. 1988a. Bacterial spot disease of stone fruits: overview of findings. Deciduous Fruit Grower 38:128-132.

Du Plessis, H.J. 1988b. Differential virulence of *Xanthomonas campestris* pv. *pruni* to peach, plum, and apricot cultivars. Phytopathol. 78:1312-1315.

Falconer, D.S. 1989. Introduction to quantitative genetics. 3rd ed. John Wiley and Sons Inc., New York.

Feliciano, A. and R.H. Daines. 1970. Factors influencing ingress of *Xanthomonas pruni* through peach leaf scars and subsequent development of spring cankers. Phytopathol. 60:1720-1726.

Fernandez, G.C.J. and J.C. Miller. 1985. Estimation of heritability by parent-offspring regression. Theor. Appl. Genet. 70:650-654.

Flor, H.H. 1955. Host-parasite interaction in flax rust - its genetics and other implications. Phytopathol. 45:680-685.

Flory, W.S. 1941. Varietal ratings of plums with reference to canker resistance. Texas Agr. Expt. Sta. Prog. Rpt. 753.

Gilbert, N.G. 1973. Biometrical interpretation. Clarendon Press, Oxford.

Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for agricultural research. 2nd ed. John Wiley & Sons, New York.

Goodman, C.A. and M.J. Hattingh. 1986. Transmission of *Xanthomonas campestris* pv. *pruni* in plum and apricot nursery trees by budding. HortScience 21:995-996.

Goodman, C.A. and M.J. Hattingh. 1988. Mechanical transmission of *Xanthomonas campestris* pv. *pruni* in plum nursery trees. Plant Dis. 72:643.

Gordon, I.L. 1979. Standard errors of heritabilities based on perennial observations, with application to Yorkshire Fog grass. Euphytica 28:81-88.

Graham, J.H., T.R. Gottwald and D. Fardelmann. 1990. Cultivar-specific interactions for strains of *Xanthomonas campestris* from Florida that cause citrus canker and citrus bacterial spot. Plant Dis. 74:753-756.

Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Aust. J. Biol. Sci. 9:463-493.

Hallauer, A.R. and J.B. Miranda Fo. 1981. Quantitative genetics in maize breeding. Iowa State Univ. Press, Ames, Iowa.

Hammerschlag, F.A. 1988a. Screening peaches in vitro for resistance to *Xanthomonas campestris* pv. *pruni*. J. Amer. Soc. Hort. Sci. 113:164-166.

Hammerschlag, F.A. 1988b. Selection of peach cells for insensitivity to culture filtrates of *Xanthomonas campestris* pv. *pruni* and regeneration of resistant plants. Theor. Appl. Genet. 76:865-869.

Hammerschlag, F.A. 1990. Resistance responses of plants regenerated from peach callus cultures to *Xanthomonas campestris* pv. *pruni*. J. Amer. Soc. Hort. Sci. 115:1034-1037.

Hansche, P.E. 1983. Response to selection, p.154-171. In: J.N. Moore and J. Janick (eds.). Methods in fruit breeding. Purdue Univ. Press, West Lafayette, Ind.

Hanson, W.E. 1963. Heritability, p. 125-140. In: W.D. Hanson and M.F. Robinson (eds.). Statistical genetics and plant breeding. NAS-NRC 982. Washington, D.C.

Hartl, D.L. and A.G. Clark. 1989. Principles of population genetics. 2nd ed. Sinauer Associates, Inc. Sunderland, Mass.

Harvey, W.R. 1990. User's guide for LSMLMW and MIXMDL pc-2 version. Mimeo, Ohio State Univ., Columbus.

Heaton, J.B. 1983. Plum: Bacterial spot cantrol. Qld. Dept. Primary Ind. Farm Note F189/Sept. 83, 2 p.

Hirano, S.S. and C.D. Upper. 1983. Ecology and epidemiology of foliar bacterial plant pathogens. Ann. Rev. Phytopathol. 21:243-269.

Huang, J. 1986. Ultrastructure of bacterial penetration in plants. Ann. Rev. Phytopathol. 24:141-157.

Hurter, N. and M.J. van Tonder. 1975. The breeding of improved Japanese plum cultivars. FFTRI, Stellenbosch Info. Bull. No. 286, 6 p.

Keil, H.L. and H.W. Fogle. 1974. Orchard susceptibility of some apricot, peach and plum cultivars and selections to *Xanthomonas pruni*. Fruit Var. J. 28:16-19.

Kempthorne, O. 1957. An introduction to genetic statistics. Iowa State Univ. Press, Ames, Iowa.

Klein, T.W., J.C. DeFries and C.T. Finkbeiner. 1973. Heritability and genetic correlation: Standard errors of estimates and sample size. Behavior Genetics 3:355-364.

Klement, Z. 1982. Hypersensitivity, p. 150-177. In:M.S. Mount and G.H. Lacy (eds.). Phytopathogenic prokaryotes, Vol. 2. Academic Press, New York.

Koonce, K.L. 1990. Mixed model least squares and maximum likelihood computer program (LSMLMW PC-1 Version). Amer. Statistician 44:49-52.

Layne, R.E.C. 1966. Susceptibility of apricots to bacterial spot infection of foliage and fruit. Plant Dis. Rptr. 50:112-115.

Leben, C. 1974. Survival of plant pathogenic bacteria. Ohio Agr. Res. and Dev. Ctr., Wooster, Ohio. Spec. Circ. 100. 21 p.

Littell, R.C. and B.G. McCutchan. 1987. Use of SAS for variance component estimation. Proc. 1986 workshop on statistical considerations in genetic testing of forest trees. South. Coop. Series Bull. No. 324. p. 75-86.

Matthee, F.N. 1968. Bacterial spot of stone fruits. 1. What do we know about this disease? Deciduous Fruit Grower 18:48-50 and 62.

Matthee, F.N. and R.H. Daines. 1968. Effects of soil and substrate aeration on stomatal activity, water diffusion pressure deficit, water congestion, and bacterial infection of peach and pepper foliage. Phytopathol. 58:1298-1301.

Millikan, D.F. and A.D. Hibbard. 1964. Bacterial spot on apricots. Plant Dis. Rptr. 48:900-901.

Milliken, G.A. and D.E. Johnson. 1984. Analysis of messy data I. Designed experiments. Lifetime Learning Pub., London.

Moffett, M.L. 1973. Bacterial spot of stone fruit in Queensland. Aust. J. Biol Sci. 26:171-179.

Nguyen, H.T. and D.A. Sleper. 1983. Theory and application of half-sib matings in forage grass breeding. Theor. Appl. Genet. 64:187-196.

Norton, J.D. 1976. 'Crimson', 'Purple' and 'Homeside' plums. HortScience 11:62-64.

Parlevliet, J.E. 1979. Components of resistance that reduce the rate of epidemic development. Ann. Rev. Phytopathol. 17:203-222.

Parlevliet, J.E. 1989. Identification and evaluation of quantitative resistance, p. 215-248. In: K.J. Leonard and W.E. Fry (eds.). Plant disease epidemiology. Vol. 2: Genetics, resistance, and management. McGraw-Hill Pub. Co., N.Y.

Popenoe, J. 1959. Relation of heredity to incidence of bacterial spot on plum varieties in Alabama. Proc. Assoc. South. Agric. Workers. 56th Ann. Conv. p. 176-177.

Quamme, H.A., F. Kappel and J.W. Hall. 1990. Efficacy of early selection for fireblight resistance and the analysis of combining ability for fire blight resistance in several pear progenies. Can. J. Plant Sci. 70:905-913.

Randhawa, P.S. and E.L. Civerolo. 1985. A detached-leaf bioassay for *Xanthomonas campestris* pv. *pruni*. Phytopathol. 75:1060-1063.

Robinson, R.A. 1976. Plant pathosystems. Springer-Verlag, Berlin.

Russell, D.M., J.B. Heaton and B.L. Topp. 1991. Japanese plum - susceptibility of cultivars to bacterial spot. Qld. Dept. Prim. Ind. Farm Note Agdex 216/633. 2 p.

SAS Institute, Inc. 1987. SAS/STAT guide for personal computers, version 6 edition. SAS Inst., Inc., Cary, N.C.

Schlotzhauer, S.D. and R.C. Littell. 1987. SAS System for elementary statistical analysis. SAS Inst., Inc., Cary, N.C.

Schultz, E.F. 1955. Rules of thumb for determining expectations of mean squares in analysis of variance. Biometrics 11:123-135.

Sherman, W.B. and P.M. Lyrene. 1981. Bacterial spot susceptibility in low chilling peaches. Fruit Var. J. 35:74-76.

Sherman, W.B. and P.M. Lyrene. 1983. Handling seedling populations, p. 66-73. In: J.N. Moore and J. Janick (eds.). Methods in fruit breeding. Purdue Univ. Press, West Lafayette, Ind.

Sherman, W.B. and P.M. Lyrene. 1985. Progress in low-chill plum breeding. Proc. Fla. State Hort. Soc. 98:164-165.

Sherman, W.B. and R.H. Sharpe. 1970. Breeding plums in Florida. Fruit Var. Hort. Dig. 24:3-4.

Simeone, A.M. 1982. Study on the susceptibility of plum cultivars to some of the principal parasites. Frutticultura 44:112-117. (English summary).

Simeone, A.M. 1990. Observation on cultivar susceptibility to natural infections of *Xanthomonas pruni* in a plum collection. Frutticoltura 52:61-63. (English summary).

Simmonds, N.W. 1979. Principles of crop improvement. John Wiley and Sons, Inc., New York.

Sprague, G.F. and L.A. Tatum. 1942. General vs. specific combining ability in single crosses of corn. J. Amer. Soc. Agron. 34:923-932.

Stall, R.E., G.M. Marco and B.I. Canteros de Echenique. 1982. Importance of mesophyll in mature-leaf resistance to cancrosis of citrus. Phytopathol. 72:1097-1100.

Steele, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics. 2nd ed. McGraw Hill Pub. Co., New York.

Swiger, L.A., W.R. Harvey, D.O. Everson and K.E. Gregory. 1964. The variance of intraclass correlation involving groups with one observation. Biometrics. 20:818-826.

Tehrani, G., W.R. Okie and D.W. Cain. 1991. Plum. In J.N. Cummins (ed.) Register of new fruit and nut varieties. List 34. HortScience 26:975-977.

Thompson, J.M. 1981. The plum industry in the southeastern United States. Fruit Var. J. 35:53-55.

Thornberry, H.H. and H.W. Anderson. 1933. Overwintering of *Phytomonas pruni* on peach. Phytopathol. 23:787-801.

Topp, B.L and D.M. Russell. 1988. Breeding early ripening Japanese plums. Acta Hort. 240:27-30.

Topp, B.L. and Sherman, W.B. 1990. Sources of bacterial spot resistance in Japanese-type plum cultivars. Fruit Var. J. 44:32-35.

Topp, B.L., W.B. Sherman and R.E. Stall. 1991. Comparison of rating methods for bacterial spot resistance in Japanese-type plum. Fruit Var. J. 45:70-74.

Topp, B.L., J.B. Heaton, D.M. Russell, and R. Mayer. 1989. Field susceptibility of Japanese-type plums to *Xanthomonas campestris* pv. *pruni*. Aust. J. Exp. Agric. 29:905-909.

USDA. 1941. Yearbook of agriculture. Climate and man. US Govt. Printing Office, Washington, D.C.

Vanderplank, J.E. 1963. Plant diseases: Epidemics and control. Academic Press, New York.

Weinberger, J.H. 1975. Plums, p.336-347. In: J. Janick and J.N. Moore (eds.). Advances in fruit breeding. Purdue Univ. Press, West Lafayette, Ind.

Werner, D.J., D.F. Ritchie, D.W. Cain and E.I. Zehr. 1986. Susceptibility of peaches and nectarines, plant introductions and other *Prunus* species to bacterial spot. HortScience 21:127-130.

BIOGRAPHICAL SKETCH

Bruce Leonard Topp was born on 19 September 1955 in Melbourne, Victoria, Australia. He is the son of Mabel Ann (nee Smith) and Leonard Victor Topp and brother of David, Helen, and Andrew. He attended Canterbury State Primary School and Camberwell State High School. He graduated from the University of Melbourne in 1977 with the degree of Bachelor of Agricultural Science (Hons.) and began work for the Queensland Department of Primary Industries as a plant breeder at the Granite Belt Horticultural Research Station, Applethorpe, Queensland. He is married to Thirley (nee Howard) and they are blessed with four children: Matthew, Keenan, Nyssa, and Lachlan. Bruce was granted study leave from the Queensland Department of Primary Industries and in January 1989 enrolled in the Fruit Crops Department, University of Florida, for the Doctor of Philosophy degree specializing in fruit breeding. Upon completion of study in the United States, he intends to return to Queensland and breed new and wonderful fruits.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. Wayne B. Sherman, Chairman
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. Paul M. Lyrene
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. Gloria A. Moore
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Robert E. Stall

Dr. Robert E. Stall
Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Kenneth H. Quesenberry

Dr. Kenneth H. Quesenberry
Professor of Agronomy

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as a partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 1992

Jack L. Fry

Dean, College of Agriculture

Dean, Graduate School